GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Founded in 1916
by
GEORGE H. SHULL
with the cooperation of

WILLIAM E. CASTLE
EDWIN G. CONKLIN
CHARLES B. DAVENPORT
BRADLEY M. DAVIS
EDWARD M. EAST
ROLLINS A. EMERSON
HERBERT S. JENNINGS
THOMAS H. MORGAN
RAYMOND PEARL

Volume 36 March, 1951 Number 2

TABLE OF CONTENTS

BEAUDRY, J. R., Seed development following the mating *Elymus virginicus* L. × *Agropyron repens* (L.) Beauv. 109

STORMONT, CLYDE, R. D. OWEN AND M. R. IRWIN, The B and C systems of bovine blood groups 134

NELSON, THOMAS CLIFFORD, Kinetics of genetic recombination in *Escherichia coli* 162

YOST, HENRY T., JR., The frequency of X-ray induced chromosome aberrations in Tradescantia as modified by near infrared radiation 176

CLARK, A. M., AND C. J. MITCHELL, Gene dosage relations for haploids and diploids of Habrobracon 185

SHENG, T. C., A gene that causes natural death in *Neurospora crassa*. 199

PUBLISHED BIMONTHLY AT LANCASTER, PENNSYLVANIA,
BY THE BROOKLYN BOTANIC GARDEN, BROOKLYN 25, NEW YORK, U.S.A.
(Date of issue, May 25, 1951)

EDITORIAL BOARD

CURT STERN, *Managing Editor*, University of California

GEORGE W. BEADLE
California Institute of Technology

WILLIAM E. CASTLE
University of California

EDWIN G. CONKLIN
Princeton University

BRADLEY M. DAVIS
University of Michigan

L. C. DUNN
Columbia University

JOHN W. GOWEN
Iowa State College

DONALD F. JONES
Connecticut Agricultural Experiment Station

M. M. RHOADES
University of Illinois

GEORGE H. SHULL
Princeton University

T. M. SONNEBORN
Indiana University (Representative of the Genetics Society of America)

L. J. STADLER
U. S. Dept. Agriculture, University of Missouri

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Chicago

Volume 36 MARCH, 1951 Number 2

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $8.00 net a year for complete volumes (January-November). Foreign postage, 50 cents additional. Single copies, $1.50 each, postpaid.

As available, volumes 1 to 6 may be had at $12.00 each, and the later volumes at $10.00 each, postpaid. The Business Manager will supply information on request as to volumes and numbers available.

Correspondence concerning editorial matters should be addressed to the EDITOR OF GENETICS, Department of Zoology, University of California, Berkeley 4, Calif.

Business Correspondence, including change of address, etc., should be addressed to 111 E. Chestnut St., Lancaster, Pa., or BROOKLYN BOTANIC GARDEN, Brooklyn 25, N. Y., U. S. A.

Remittances should be made payable to GENETICS, INC.

Entered as second-class matter, August 31, 1922, at the postoffice at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L.&R., authorized January 9, 1932. Application for re-entry at Lancaster, Pa., pending.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

SEED DEVELOPMENT FOLLOWING THE MATING ELYMUS VIRGINICUS L. × AGROPYRON REPENS (L.) BEAUV.[1]

J. R. BEAUDRY

Department of Genetics, University of Wisconsin, Madison, Wisconsin[2]

Received May 15, 1950

FAILURE of seed formation in wide crosses of Gramineae cannot be explained on the basis of the theory of somatoplastic sterility (COOPER and BRINK 1944; BRINK and COOPER 1944; THOMPSON and JOHNSTON 1945). This theory was formulated to account for the difference in early seed development of self- and cross-fertilized *Medicago sativa* (BRINK and COOPER 1940). Evidence supporting the explanation was afforded by subsequent investigations on seed development of different species of Nicotiana (COOPER and BRINK 1940), and of races of Lycopersicon (COOPER and BRINK 1945) following controlled differential pollinations. One of the main facts on which the theory is based is a hyperplasia of the integumentary tissue of the seed, starting at a point opposite the end of the vascular bundle. Evidence of hyperplasia, however, is lacking in wide crosses of Gramineae but novel factors, expressed in the behavior of the antipodals, were found to be of significance for the interpretation of the events culminating in the death of these seeds (BRINK and COOPER 1944).

The large number of antipodals and their persistence after the occurrence of double fertilization are prominent features of the megagametophyte in the Gramineae. When *Hordeum jubatum* was selfed, BRINK and COOPER found that four hours after pollination its antipodals had enlarged considerably, their size then being more than six times as large as at the time of pollination. When, however, *Hordeum jubatum* was fertilized by sperms of *Secale cereale,* enlargement of the antipodals was limited to about 65 percent of their initial size. The antipodals persisted longer in crossed seeds, and some of their nuclei underwent mitosis while the nuclei remained quiescent in selfed seeds. A second cross, *Hordeum vulgare* × *Secale cereale,* provided corroborative evidence.

These observations, and other considerations, led BRINK and COOPER to ascribe to the antipodals an important role in seed formation in the Gramineae: "The prominent development of the antipodals athwart the nutrient stream in *Hordeum jubatum,* the rapid enlargement of these cells at fertilization associated with a corresponding increase in size of the embryo sac and the extraordinary changes through which the nuclei and chromosomes pass all

[1] Adapted from a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Wisconsin. Paper No. 424 from the Department of Genetics, University of Wisconsin.

[2] Present address: Institut de Biologie générale et de Zoologie, Université de Montréal, Montreal, Que., Canada.

GENETICS 36: 109 March 1951.

Second Printing 1969 / University of Texas Printing Division, Austin

point to an active participation of the antipodals in the transformation of the ovules into a young seed. The resemblance of the nuclei of the antipodals of this class to those of glandular cells in general, noted by earlier investigators, definitely suggests a metabolic role. Substances necessary for the initial growth of the endosperm are probably built up in the antipodals from materials conveyed thence by the vascular bundle, and then secreted into the endosperm mother cell. The endosperm evidently becomes the dynamic center of the entire structure since growth stops upon its breakdown in the hybrid seed. The antipodals function for a brief period only, but since this period falls at the crucial stage of endosperm initiation their influence may be decisive for the whole subsequent course of seed development."

THOMPSON and JOHNSTON (1945) were led to a different conclusion from a similar study of seed failure following the cross *Hordeum vulgare* × *Secale cereale*. They report that "The behaviour of the antipodal cells is not sufficiently different from normal to be of significance."

The present work is a re-examination of the events leading to seed death following a wide cross in the Hordeae.

MATERIALS AND METHODS

Representatives of a number of genera of the tribe Hordeae were grown in a greenhouse and crossed in various combinations in a search for an additional mating in which double fertilization occurs but is followed by breakdown of the seed before completion of its development. It was found that seed development was initiated when *Elymus virginicus* L. (n = 14) was pollinated by *Agropyron repens* (L.) Beauv. (n = 21) but that the seeds produced never reached a germinable condition. The cross *E. virginicus* × *A. repens* was thus selected for detailed study.

E. virginicus plants, growing in a uniform colony at Albany, Wisconsin, were collected, potted in ten-inch pots, and grown in the open. The lowermost floret of the spikelets forming the five or six central pairs of spikelets in a spike were emasculated when the anthers began to turn yellow, the other florets and spikelets being excised. Certain of the ovaries of the emasculated florets were selfed or pollinated with pollen of other plants of the same species. The seed resulting from these intraspecific matings will be referred to as control. Other *E. virginicus* ovaries were pollinated with pollen of *A. repens* obtained from a colony of that species growing near Madison, Wisconsin. The seeds arising from this intergeneric cross will be referred to as hybrid. Unpollinated ovaries of *E. virginicus* were collected at the time of pollination. Control and hybrid seeds were collected at 4, 6, 9, 12, 18, 24, 36, 48, 60 hours and 3, 4, 5, 9, 15, and 30 days after pollination. The seeds were prefixed in Carnoy's fixative and then transferred to a modified Nawaschin's fixative. After fixation, the seeds were thoroughly washed in running water, dehydrated in tertiary butyl alcohol as recommended by JOHANSEN (1940) and embedded in paraffin. The seeds were cut at 15 micra in thickness, and the sections were mounted serially. Most of the serial sections were stained for two hours in Feulgen's fuchsin sulfurous reagent (STOWELL 1945) after a

previous hydrolysis of 25 minutes in normal HCl at 60°C. Seeds used to control the specificity of the Feulgen reaction for desoxyribonucleic acid were stained simultaneously without previous hydrolysis. A 0.01 percent solution of fast green in 95 percent alcohol was often used as a counterstain. Complete serial sections were usually stained 30 at a time, 15 of them frequently representing three stages of seed development of *E. virginicus* and the remainder the three comparable stages of the hybrid.

Two series of hybrid and control seeds, one treated with ribonuclease and the other not thus treated were stained for 25 minutes under identical conditions with Unna's methyl green–pyronin mixture (BRACHET 1940, 1942). The stain mixture was prepared as recommended by LA COUR (1947). The seed sections treated with ribonuclease were kept for three hours in a solution of 10 mg of ribonuclease in 50 cc of distilled water at 60°C, while the non-treated seed sections were kept in distilled water at 60°C during the same period of time. The ribonuclease used was obtained from the Armour Laboratories, Chicago, Illinois. Age of the seeds is counted from the time of pollination.

FERTILIZATION AND SEED DEVELOPMENT IN ELYMUS VIRGINICUS

The ovule of *E. virginicus* is anatropous and bitegumentary, each integument being two cell-layered. The vascular bundle, which enters the ovary at its base, runs medially in the ventral ovarian wall up to the region opposite the antipodals. The mature gametophyte includes, in addition to its egg, two synergids, two polar nuclei, and a mass of antipodals varying in number from 19 to 31 (figs. 1 and 21).

One male gamete nucleus is already appressed to the egg nucleus, and the other to one or both of the polar nuclei at four hours, but fertilization has not yet occurred. The primary endosperm nucleus divides at about six hours. The endosperm is free-nucleate during the early stages of development. The nuclei lie in cytoplasm which gradually accumulates at the periphery of the expanding endosperm mother cell and encloses a large central vacuole. The nuclei divide synchronously in rhythmic mitotic waves starting in the micropylar region. This pattern of mitosis leads to a geometric increase in the number of nuclei. The endosperm contains a modal number of two nuclei at nine hours, eight at 18 hours, and 32 at 36 hours. The average number of nuclei is 128 at 48 hours and the endosperm is still multinucleate. The micropylar pocket of the endosperm becomes cellular at 60 hours (fig. 3). Thereafter, the geometric pattern of growth is gradually modified. Part of the endosperm is still multinucleate at three days, but by four days the tissue is completely cellular and has entirely filled the central lumen. The first signs of differentiation of the aleurone layer can be seen in the region adjacent to the embryo at the latter stage. Relative to the other endosperm cells, the aleurone cells are smaller and more regular in shape, tending to be rectangular. Their cytoplasm is also denser. Starch deposition in the endosperm starts shortly after the aleurone is initiated.

There is a considerable lag between the completion of fertilization of the

polar nuclei and that of the egg. Actual fusion of the male gamete nucleus with the egg nucleus occurs between 24 and 36 hours. The embryo develops more slowly than the endosperm. The proembryo contains an average of ten cells at 60 hours whereas the accompanying endosperm, which is partly cellular and partly multinucleate, contains almost 600 nuclei. The earliest signs of embryo differentiation appear at four days.

The antipodals are almost terminal in position in the mature gametophyte. Their initial position is modified soon after syngamy by enlargement of the endosperm mother cell. The latter expands in all directions but especially in length, beyond the antipodals. The position of the antipodals is thus changed from terminal to median lateral. The antipodal cells including their nuclei enlarge slowly and gradually as the endosperm mother cell expands. The cytoplasm of these cells concurrently becomes more and more vacuolate. The entire mass of antipodals reaches a maximum size at 48 hours. Twelve hours later, however, these cells have completely regressed, being then reduced to flattened remnants compressed between the nucellus and the developing endosperm (fig. 2).

Feulgen positive threads, traceable to nuclei, occur in positions suggesting lines of flow toward the proembryo in 60-hour and older seeds (fig. 3).

The tips of the pollen tubes of *E. virginicus* and *A. repens* take a reddish color when young seeds are treated with the Feulgen fuchsin sulfurous reagent without previous hydrolysis. The staining reaction of these pollen tube tips cannot thus be considered as a positive Feulgen reaction, indicative of the presence of desoxyribonucleic acid. The nucleus of the egg and of the polar nuclei of *E. virginicus* are almost Feulgen negative in the mature gametophyte, while the antipodal nuclei are strongly Feulgen positive (fig. 1). The first endosperm nuclei produced are very faintly Feulgen positive. Stainability of the endosperm nuclei increases subsequently until 36 hours when it appears to reach a maximum which is maintained thereafter.

FERTILIZATION AND SEED DEVELOPMENT IN ELYMUS VIRGINICUS × AGROPYRON REPENS

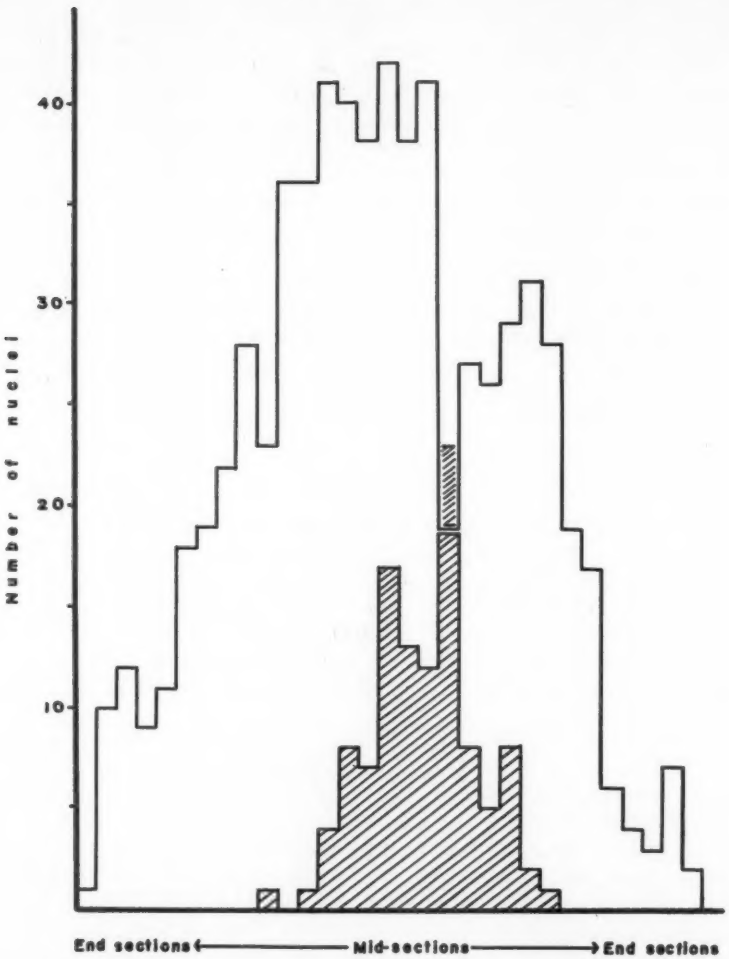
Fertilization of the polar nuclei often occurs earlier in the hybrid than in the normally mated *E. virginicus*. It has been observed twice at four hours, and two endosperm nuclei were seen in one seed at six hours. Fusion of polar nuclei and fertilization occur simultaneously in the hybrid seeds. The three nuclei enter prophase while still conjoined as a triunitary group, each entity being well defined by its own membrane. Thereafter growth of the endosperm, in terms of nuclei, proceeds rapidly. The modal number of nuclei at 9, 18, and 36 hours after pollination are 2, 18, and 128, respectively. The average number of nuclei is 514 at 48 hours. Cell formation first appears in the micropylar pocket at 60 hours (fig. 8), when the endosperm contains slightly more than a thousand nuclei. All endosperm nuclei appear normal in size or shape until 48 hours.

Endospermic irregularities, analogous to those described in interspecific

crosses of Avena (KIHARA and NISHIYAMA 1932) of Triticum (BOYES and THOMPSON 1937), and in the intergeneric crosses *Hordeum jubatum* × *Secale cereale* (COOPER and BRINK 1944), and *H. vulgare* × *S. cereale* (COOPER and BRINK 1944, THOMPSON and JOHNSTON 1945), were observed in one of the seeds fixed at 48 hours after pollination (figs. 4, 6, and 22), and in all the seeds collected at later stages. The endosperm nuclei in normal seeds are oval or ovo-globular in shape. Irregular interphase endosperm nuclei in hybrid seeds may be more or less misshapen. Sometimes they are greatly elongated, the long axis being several times that of the normal. Some nuclei show abrupt and long intercalary or terminal constrictions (fig. 5). Others gradually taper at one end. Still others are branched. Dumbbell-shaped nuclei are very common (figs. 6 and 22). The shape of certain nuclei is so irregular as to defy description. Nuclear size also varies widely. At one extreme are micronuclei and at the other are giant nuclei (fig. 4) which are several times the volume of a normal nucleus. These enormous nuclei often contain eight or more nucleoli. Finally, some interphase nuclei having none, one or more of the above characteristics, stain so intensely with the Feulgen reagent and with methyl green–pyronin that they stand in sharp contrast with normally staining ones (fig. 7). The kinds of abnormalities observed in nuclei undergoing mitosis suggest interpretation of the various configurations observed in interphase nuclei. Chromosome bridges uniting anaphasic or telophasic groups of chromosomes undoubtedly lead to the formation of dumbbell-shaped nuclei (COOPER and BRINK 1944). Multipolar spindles are probably responsible for the occurrence of branched nuclei; lagging chromosomes for micronuclei; repeated failure of spindle movement for highly compound metaphase plates and giant nuclei; unequal distribution of chromosomes for both micro- and macronuclei.

The distribution of irregular nuclei in the endosperm is worthy of mention. Examination of the longitudinal dorsiventral sections of the 60- and 72-hour seeds, serially arranged on the slides, reveals that the irregular nuclei are not distributed at random, but are clustered in the mid-sections (textfig. 1). If the irregular nuclei were distributed at random in the tissue, there should be a positive correlation between the numbers of regular and irregular nuclei in each section. The actual numbers of irregular nuclei found in successive serial sections of six hybrid seeds collected at 60 or 72 hours were compared, by means of a chi-square test, with corresponding expected numbers of irregular nuclei calculated on the basis of the above-mentioned hypothesis. This test shows that there are highly significant differences between the actual and expected distributions of irregular nuclei ($\chi^2 = 217.5$; P (33 d.f.) = very low). The irregular nuclei are further localized in their distribution as may best be seen in longitudinal mid-sections of the seeds. They appear first around the antipodals and subsequently in the other parts of the endosperm.

The endosperm cytoplasm itself displays peculiarities not encountered in the normal seed. A large-sized canal is evident in the vicinity of the embryo when methyl green–pyronin staining is used. This canal, almost completely



TEXTFIGURE 1.—Number of regular (white histogram) and irregular (cross-hatched histogram) endosperm nuclei in each section of a hybrid seed, fixed 60 hours after pollination, cut at 15 micra in longitudinal, dorsiventral and serially arranged sections. The irregular nuclei are clustered in the mid-sections.

devoid of stainable material, runs more or less parallel to the longitudinal axis of the seed, extends through several sections and is present from 60 hours onwards (figs. 8 and 24). Another observation which is reported for the first time concerns the presence of globular bodies in the central lumen of the endosperm (figs. 8 and 24; 10 and 23). These bodies are particularly abun-

dant in the micropylar end. They have been noted at 60, 72, and 96 hours. They vary considerably in size. They appear to comprise an inner core of cytoplasm staining with pyronin, surrounded by a thin, non-staining, hyaline matrix. A few of these globules enclose one or more Feulgen-negative corpuscles, staining more intensely with pyronin than the cytoplasm in which they are embedded (figs. 10 and 23). The origin of these globules can be traced back to the endosperm itself, from which they arise by a process like budding. The globules first appear as segments of a sphere and then as a hemisphere bulging out from the cytoplasm. Later stages are seen as spheres still attached to the cytoplasm by a stalk. The globules remain spherical after release and collect in the large central vacuole. A nucleus is commonly present at their point of origin.

A further peculiarity of the endosperm of the hybrid seed is that from 36 to 48 hours, parts of the endosperm cytoplasm stain much more intensely with the pyronin of the methyl green–pyronin mixture than their counterparts in a normal seed. (Compare the intensity of staining in the micropylar pockets of the sections shown in figures 8 and 9; 24 and 25). The spatial distribution of these deeply red-staining parts and the changes which occur in their distribution as the seed ages correspond to those of the irregular nuclei.

The endosperm eventually becomes entirely disorganized and may completely disintegrate. The walls of the seed collapse (fig. 11) and death of the seed ensues. The time at which endosperm disorganization occurs varies from seed to seed. Many seeds may be completely or almost completely devoid of endosperm at three days. Nine-day-old seeds, however, have been examined in which a fair amount of endosperm was still present, although highly irregular in appearance. Small fragments of endosperm, isolated along the length of the seed between the collapsed walls and still containing mitotically active irregular nuclei, show that the endosperm nuclei retain their ability to divide to the last.

Although the male gamete is near the egg nucleus at four hours, it still retains its individuality at 18 hours. The first division of the zygote was taking place in one exceptional ovule at this time. Fertilization of the egg is completed between 18 and 24 hours, since two-celled proembryos were always present at 24 hours. The proembryo is about 14-celled and is apparently normal at 60 hours, even though endosperm irregularities are present in every hybrid seed. The proembryo continues to grow for a variable period of time after the breakdown of the endosperm. In one instance death had already occurred at four days, but it seemed that the proembryo had been crushed by the collapse of the seed walls, which were closely appressed to each other. The proembryo was not crushed by the collapsed walls in most seeds examined, however, but left in a pocket, with enough space to allow for considerable further growth. Proembryos, enclosed in seeds completely devoid of endosperm, continue to grow by mitosis (fig. 12). The supply of nutrients to support growth under such circumstances probably comes from the nu-

cellus, which is still fairly abundant on the ventral side of the seed when collapse occurs. It seems that when both the endosperm and nucellus have disappeared, death of the embryo soon follows, because in all the seeds reaching this stage the embryos were disintegrating. The embryos may show definite signs of differentiation before dying.

The antipodals enlarge slowly and gradually after syngamy, until 60 hours. They are usually still present at three days but clearly in a state of disorganization. They are apparently normal until 18 hours when the nucleus of one or more shows signs of activity expressed by the condensation of the chromatin into chromosomes and the division of the nucleus. Some late prophase stages show the occurrence of polytenic chromosomal aggregates, *i.e.*, of separate groups made up of more than two strands (fig. 17). It has been possible to count 14 such aggregates, a number corresponding to the haploid number of chromosomes in *E. virginicus*. Other antipodal nuclei show more than a hundred apparently free and uniformly scattered chromosomes. One such nucleus was seen which had separated into four somewhat unequal groups of chromosomes, the groups being still united by spindles on which laggards were present (fig. 15). Polynucleate antipodals were commonly observed. These signs of activity of the antipodal nuclei can be found in nearly all the seeds after 18 hours (figs. 13, 15, 17, 18 and 26; 19 and 27). Antipodal nuclei often assume an irregular shape and contain a large number of nucleoli (fig. 14).

One antipodal nucleus was seen connected to an endosperm nucleus (fig. 16). The shape of the nucleoli of these two nuclei, revealed by careful focusing, is modified in such a way that a long-pointed lobe extending from each nucleolus reaches the point of union of the two nuclei.

COMPARISON OF FERTILIZATION AND SEED DEVELOPMENT IN E. VIRGINICUS AND E. VIRGINICUS × A. REPENS

Comparison of fertilization and development in control and hybrid seeds reveals several differences. *E. virginicus* polar nuclei are fertilized earlier by *A. repens* sperm than by the sperm of their own species. The male gamete nucleus of *E. virginicus* is conjoined with the polar nuclei at four hours but still retains its individuality. Completion of fertilization, as identified by the first mitosis of the primary endosperm nucleus or the two nuclei resulting therefrom, was observed in a few hybrid seeds at this age.

The average number of nuclei present in the endosperm at definite periods after pollination is given in table 1. These numbers are lower for *E. virginicus* than for the cross at all stages except at twelve hours where the average number of endosperm nuclei in the hybrid seed is 2.67 as compared to four in the control seed. The number 2.67, however, is based on counts made from three seeds only. A large number of hybrid seeds, fixed 12 hours after pollination, were prepared for study but all those examined except these three were unfertilized. It is possible that if counts could have been made on a larger number of seeds that this value, representative of the 12-hour stage in

TABLE 1

Average number of endosperm nuclei in E. virginicus and E. virginicus × A. repens.

Time in hours	*E. virginicus*	*E. virginicus × A. repens*
4	0.00	0.29
6	0.83	1.20
9	0.90	2.14
12	4.00	2.67
18	6.00	9.00
24	4.80	20.57
36	30.86	128.00
48	128.00	514.00
60	590.00	1006.67
72	944.00	1742.33

the cross, would be higher than that for *E. virginicus*. An explanation needs also to be given for the value of 4.80, representing the average number of nuclei at the 24-hour stage in the endosperm of the control seeds. It will be noted that this value is lower than that of the 18-hour stage in the same seeds, which is 6.00. One of the seeds studied at 24 hours contained eight endosperm nuclei. Each of four others contained four nuclei in advanced stages of mitosis. Since none of these nuclei had yet reached the stage of telophase when formation of the nuclear membrane takes place, *i.e.*, the stage at which the daughter nuclei can be considered independent, each was counted as a single unit. The average number of nuclei in these seeds, however, dynamically speaking, is closer to eight than 4.80.

The data given in table 1 have been plotted in a semilogarithmic graph (textfig. 2). The endosperm curves show the precedence of the hybrid endosperm over that of the control. A comparison of the slopes of these curves shows that at 24 hours and thereafter, the rate of growth of the endosperm, in terms of number of nuclei, is the same in the control and in the cross. The endosperm of the hybrid seed has thus established its precedence in earlier stages.

The part of the ventral layer of the endosperm which is appressed to the

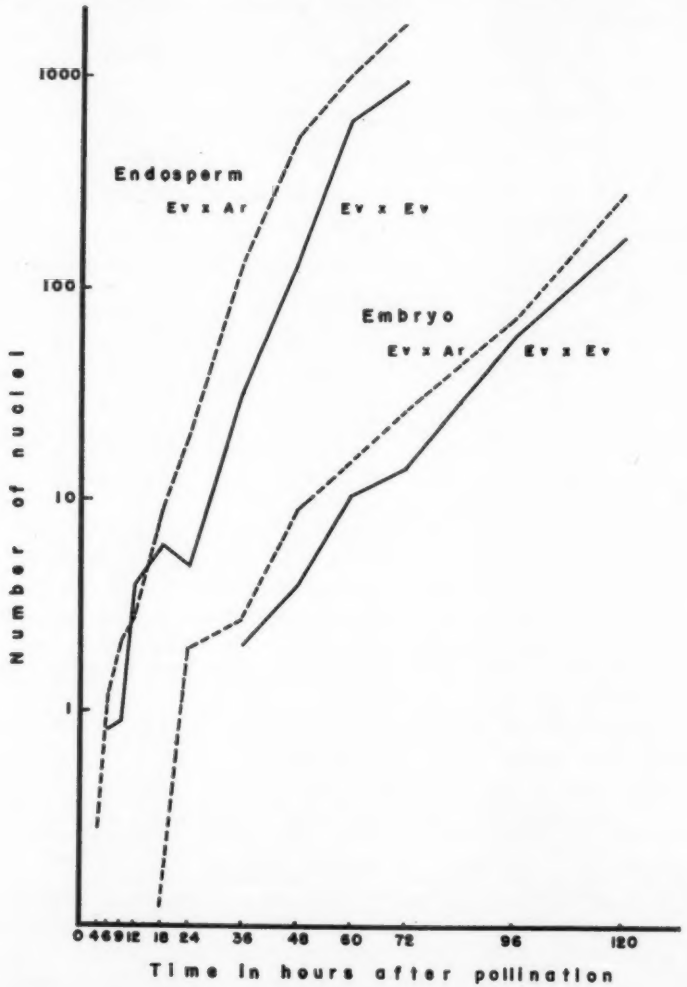
TABLE 2

Average total number of regular and irregular endosperm nuclei, and percentage of irregular nuclei in E. virginicus and E. virginicus × A. repens.

Time in hours	*E. virginicus*		*E. virginicus × A. repens*		
	Average no. of nuclei	Irregular nuclei	Average no. of nuclei	Irregular nuclei	Percent of irregular nuclei
0–36	...	...	...	...	0.00
48	128.00	0.00	514.50	1.75	0.34
60	590.00	0.00	1006.67	40.33	4.01
72	944.00	0.00	1742.33*	195.00*	11.19*
96				frequent collapse	

*From counts made on part of the seeds studied. The endosperm was disorganized in several seeds studied at this stage.

antipodals and extends therefrom to the micropylar pocket is usually thicker in control seeds than in hybrid seeds at 60 hours. The whole micropylar pocket and part of the above-mentioned endosperm layer is cellular at 60 hours in *E. virginicus*, but cell formation is then just being initiated in the



TEXTFIGURE 2.—Early growth of the endosperm and young embryo in *E. virginicus* (solid line) and *E. virginicus* × *A. repens* (broken line). The logarithms of the average number of nuclei are plotted against time in hours after pollination.

endosperm of the hybrid seeds. Endosperm nuclei of the hybrid seeds begin to depart widely from control seed endosperm nuclei at 48 hours by manifesting irregularities in size, shape, number of nucleoli, and staining reaction. Table 2 summarizes the comparative data on numerical nuclear differences between control and hybrid seeds. The average percentages of irregular nuclei in hybrid seeds are 0.34, 4.01, and 11.19 at 48, 60, and 72 hours, respectively. The endosperm of several hybrid seeds studied, however, was already disorganized at 72 hours. It seems that the events leading to the disorganization of the endosperm are precipitated between 60 and 72 hours.

Certain portions of the endosperm cytoplasm of hybrid seeds stain much more intensely with pyronin than do their counterparts in control seeds at 36 hours and onward. The endosperm of the hybrid seeds is further characterized by the capacity to produce the previously mentioned globules.

The occurrence of the many irregularities of the endosperm in the hybrid seed is followed by the complete disorganization of this tissue and its disappearance from the seed. The endosperm of control seeds follows the usual course of development as described for many Hordeae (SCHNARF 1929 and 1931).

A. repens sperm fertilizes the egg of *E. virginicus* more rapidly than does *E. virginicus* sperm. Fertilization of the egg may be completed at 18 hours in hybrid seeds, while in control seeds it is completed between 24 and 36 hours. Data concerning the growth of the two corresponding kinds of proembryos or embryos are presented in table 3 and plotted in textfigure 2.

The slopes of the embryo curves in textfigure 2 show that the hybrid embryo simply maintains in later stages of growth an initial advantage acquired in early stages. The hybrid embryo lags behind the normal embryo after five days and may or may not differentiate. It dies eventually, while the embryo of control seeds completes normal development. There is no apparent difference between the antipodals of the control and hybrid seeds until 18 hours. Starting at this stage, however, many antipodal nuclei in hybrid seeds exhibit signs of nuclear activity. This phenomenon has never been observed in the antipodals of normal seeds. The antipodals persist longer in hybrid than in control seeds.

TABLE 3

Average number of cells in the proembryo or embryo of E. virginicus and E. virginicus × A. repens.

Time in hours	*E. virginicus*	*E. virginicus* × *A. repens*
12	0.00	0.00
18	0.00	0.12
24	0.00	2.00
36	2.07	2.75
48	4.00	9.00
60	10.43	14.67
72	14.17	25.00
96	57.17	71.50
120	166.50	271.60

DISCUSSION

All who have studied the problem of failure of seed formation in wide crosses of Hordeae agree that the immediate cause of seed failure is breakdown of the endosperm. BRINK and COOPER excepted, their views concerning the primary cause of the phenomenon center around the original hypothesis of WATKINS (1927), namely, that of an altered chromosome balance in the endosperm nucleus. WATKINS, working with species of Triticum differing in chromosome number, obtained a high percentage of germinable seeds when the female parent had a higher chromosome number than the male parent (high × low cross) and a low percentage of germinable seeds in the reciprocal cross (low × high cross). He concluded that this difference in the degree of success of the reciprocal crosses cannot be attributed to the chromosome constitution of the embryos which is the same in both cases. The chromosome constitution of the endosperm, however, varies with the direction of the cross, and germination is good when the extra chromosomes are present in the diploid state, and it is poor when they are present in the haploid condition. WATKINS did not study seed development.

This hypothesis cannot be of general application as shown by the fact that in crosses between 14- and 21-chromosome species of Avena made by KIHARA and NISHIYAMA (1932) 73 to 100 percent of the seeds obtained in low × high combinations were viable. These authors also made a comparison of early seed development in *A. strigosa* (n = 7) × *A. fatua* (n = 21), in the reciprocal cross, and in the two selfed parents. The rate of growth of the hybrid endosperms was compared with that of the endosperms in the selfed mother plants. They found that it was faster in the low × high combination and slower in the high × low combination than in the mother plant selfed. KIHARA and NISHIYAMA concluded that an excessive number of chromosomes, brought in by the male gametes in low × high crosses, exerts too strong a stimulus on the endosperm nuclei. On the other hand, the stimulus imparted by a male gamete having a chromosome number lower than normal (high × low crosses) is too weak and growth of the endosperm is slowed down. The data of WAKAKUWA (1934) show that this hypothesis cannot be accepted. The growth of the endosperm in *Triticum spelta* × *T. polonicum*, *T. polonicum* × *T. aegilopoides*, and *T. spelta* × *T. aegilopoides* (all high × low crosses) is more rapid than that of the endosperm of the respective mother plant selfed. Moreover, in *T. polonicum* × *T. spelta* (low × high) the endosperm development is slower than in the mother species selfed. BRINK and COOPER (1947) also pointed out that the rate of endosperm growth is lowered rather than raised in $2n \times 4n$ Lycopersicon crosses in comparison with the $2n \times 2n$ control.

WAKAKUWA (1934) differs from all other workers in assigning an active role to both the endosperm and the embryo in seed failure. His basic concept, however, as to the cause of seed failure is also one of chromosome or genom balance between the male gamete nucleus and the polar nuclei on the one hand, and the male gamete and the egg on the other hand. In the high × low

cross the endosperm would develop more rapidly than in the mother plant selfed, and in the low × high cross the reverse would occur. Abundant evidence from the investigations of KIHARA and NISHIYAMA (1932), BOYES and THOMPSON (1937), BRINK and COOPER (1944), and the present work show that the endosperm development may be slowed down in high × low crosses and speeded up in low × high crosses. Nor does WAKAKUWA's generalization hold concerning the effect of the numerical relationship of the chromosomes between the male gamete and the egg on growth of the embryo when tested against the available data.

BOYES and THOMPSON (1937) discussed various causes which have been suggested to explain endosperm breakdown and concluded that any explanation offered must include the concept of chromosome unbalance in the endosperm.

THOMPSON and JOHNSTON (1945) attribute the breakdown of the endosperm, in the seed arising from the cross *Hordeum vulgare* × *Secale cereale*, to the constitution of the endosperm itself or to some kind of reaction between the mother plant and the hybrid endosperm. Since the endosperm of *H. vulgare* selfed and that of the hybrid have the same number of chromosomes, this concept must be considered mainly qualitative. BRINK and COOPER (1944) rejected the idea that two diverse genom- should impair mitosis when brought together in a hybrid nucleus. They have pointed out the regularity of mitosis in embryos of hybrid seeds, and supported their contention by raising an intergeneric embryo to the adult plant stage after removal from an immature seed bound to die (BRINK, COOPER and AUSHERMAN 1944). THOMPSON and JOHNSTON do not accept this argument. They point out that chromosomal and genic conditions are not the same in the endosperm as in the embryo. "When there are two barley chromosomes or genes of a particular kind to one of rye (in the endosperm) there may be present an unbalanced condition that does not occur when there is one of each as in the embryo." This view might apply to the cross *H. vulgare* × *S. cereale* but does not explain the absence of mitotic irregularities in the embryos of *H. jubatum* × *S. cereale*, of *Avena fatua* × *A. strigosa* and reciprocal, of *Elymus virginicus* × *Agropyron repens*, and other similar crosses in which the embryo as well as the endosperm is unbalanced.

Evidently WATKINS' hypothesis of numerical balance and its various modifications cannot account for the facts of seed development in the Hordeae. The evidence presented here supports BRINK and COOPER's hypothesis (1944) that the irregularities of endosperm development are induced by the abnormal behavior of the antipodals under the stimulus of a foreign sperm in its association with the polar nuclei.

The sperms of *E. virginicus* and *A. repens* are the only differential used in the production of the control and hybrid seeds, and that of *A. repens* must be considered as the primary cause of all the developmental peculiarities which distinguish the hybrid seeds from the control seeds of *E. virginicus*. Since the abnormal behavior of the antipodals is the only significant deviation from

normal growth occurring before the advent of endosperm disturbances, it must be deduced that the triunitary entity formed by the sperm of *A. repens* and the two polar nuclei of *E. virginicus* imparts an abnormal stimulus to the antipodals of *E. virginicus*. The antipodals which thus become modified in turn react upon the endosperm, altering the subsequent development of this tissue.

The antipodals are believed to play a role in the nutrition of the endosperm. Several investigators have provided evidence pointing to this function. The literature on the subject has been reviewed by BRINK and COOPER (1944). These authors emphasize the fact that the antipodals are located across the path of the nutrients carried into the seed. The observation has been made by all investigators of seed development in the Hordeae that the endosperm adjacent to the antipodals is always thicker than elsewhere in early stages of development of the seed. In the cross *Avena fatua* × *A. strigosa* (KIHARA and NISHIYAMA 1932) endosperm regeneration, after the destruction of this tissue, starts near the antipodals. Additional evidence strengthening the above deduction is provided by the fact that in *Elymus virginicus* × *Agropyron repens* many of the endosperm nuclear irregularities are first located in the vicinity of the antipodals. It has been shown also that the irregular endosperm nuclei are not distributed at random but concentrated about the longitudinal midsections of the seed. This indicates the existence of a center which acts on the endosperm nuclei in disturbing their physiology.

Absence of signs of nuclear activity in the antipodals of control seeds and their presence in the hybrid seeds show that the antipodals of *E. virginicus* are abnormally stimulated in hybrid seeds. BRINK and COOPER (1944) have suggested that the action of the foreign sperm "proceeds from the polar nuclei and their associated sperm. Just prior to fertilization the two polar nuclei are closely adjacent to the egg; but immediately thereafter the two associated nuclei move to a position between the egg and the antipodals, approaching the latter." The same observation has been made in the present study. It can be added that one of the two nuclei resulting from the division of the primary endosperm nucleus is always found near the antipodals. A mechanism for the transmission of the stimulus to the antipodals may thus exist.

The antipodals are thus considered the intermediaries through which the unusual association formed by a sperm and polar nuclei foreign to each other acts in initiating the endosperm phenomena which lead to the death of the seed.

The concept that the antipodals act as a secretory organ seems to be well established. It is now accepted by most students of the subject. This function of the antipodals suggests that they may be responsible for the presence of the heavily staining material, extending from the vicinity of the antipodals to the whole micropylar pocket in the endosperm cytoplasm of hybrid seeds, but the possibility that this material arises from the endosperm itself cannot be ruled out with the evidence at hand. The distribution of this heavily staining

material, however, and the occurrence of a peculiar type of mitosis in the antipodals seem to indicate a relationship between the two. PAINTER (1943) has reported that the initiation of nuclear activity in the tapetum of *Rhoeo discolor* is accompanied by the presence of large quantities of basophilic material, in the cytoplasm of the tapetal cells, which is subsequently released and accumulates in the cytoplasm of the maturing microspores. This material, like that discussed here, stains intensely with pyronin. A further parallel may exist between the behavior of the tapetum of *Rhoeo discolor* and that of the nuclei of the antipodals of hybrid seeds. The divisions occurring in the tapetum of Rhoeo are endomitotic as in the tapetal cells of other plants (WITKUS 1945). The nuclear configurations observed in the antipodals of the *Elymus virginicus* × *Agropyron repens* seed show that endomitosis is occurring or has taken place previously. Individual chromatic groups are seen which contain numerous strands. Nuclei containing more than a hundred chromosomes have been observed whereas the original number is 14. It must be noted that at least some of these endomitotic nuclei divide into two and possibly more nuclei.

These signs of post-syngamic activity of the nuclei of antipodals in wide crosses of Hordeae seem to be of more than casual occurrence. COOPER and BRINK (1944) have observed them in their extensive studies of the intergeneric cross *Hordeum jubatum* × *Secale cereale*. Other intergeneric crosses were made during the initial phase of the investigation reported here. Cytological observations were made on the seeds of two crosses in which seed formation takes place: *Hystrix patula* × *Agropyron repens* and *Hordeum bulbosum* × *Secale cereale*. Similar post-syngamic activity of antipodal nuclei was observed in both cases (see fig. 20 for *H. patula* × *A. repens*). It was not reported however, in *Hordeum vulgare* × *Secale cereale* (BRINK and COOPER 1944) but a rather limited number of seeds have been studied in this case (authors' communication). No mention of it is made in the reports of KIHARA and NISHIYAMA (1932), WAKAKUWA (1934), BOYES and THOMPSON (1937). THOMPSON and JOHNSTON (1945) did not observe it in their study of *H. vulgare* × *S. cereale*. It must not be forgotten that all these authors, THOMPSON and JOHNSTON excepted, have paid very little attention to the antipodals, and that all of them have studied very few of the earlier stages of seed development during which observable signs of nuclear activity are present in the antipodals of *Elymus virginicus* × *Agropyron repens, Hordeum jubatum* × *Secale cereale, Hystrix patula* × *A. repens,* and *Hordeum bulbosum* × *S. cereale.*

This post-syngamic activity of the nucleus of antipodals does not occur in normal seeds of *Elymus virginicus,* and it must be considered as an abnormal reaction arising in hybrid seeds because of the presence of a foreign sperm. The physiology of the antipodal cell, however, may have been disturbed in a more generalized way before its appearance, but it is the first clear sign of an abnormal condition.

The presence of polytene chromosomes in the antipodals of hybrid seeds

shows the occurrence of endomitosis. The question arises as to whether endomitosis is a reaction caused by the wide cross or a phenomenon of regular occurrence during the ontogeny of the antipodals of *E. virginicus* which is simply revealed by the post-syngamic activity of the antipodal nucleus following the wide cross. The nuclei of the antipodals, in the mature gametophyte, are much larger than the other gametophytic nuclei and the nuclei of the diploid tissues forming the mature ovule in the Hordeae. In addition, when stained with the Feulgen reagent, their purple color is strikingly deeper than that of these other nuclei, especially those of the mature gametophyte. These two facts favor the second interpretation. Further work will be done on this problem and to determine whether the post-syngamic nuclear phenomena observed in the antipodals of hybrid seeds can themselves be classified as endomitotic or better as mitosis following a period of endomitosis.

The hybrid endosperm nuclei which take a much deeper color than other hybrid endosperm nuclei or endosperm nuclei of control seeds, after staining with the Feulgen reagent, contain excessive amounts of desoxyribonucleic acid.

The endosperm of the hybrid seed becomes a highly disorganized tissue as evidenced by the above-mentioned irregularities of its nuclei, cytoplasm, and general morphology. It finally breaks down and disappears from the seed. The embryo then dies of starvation. Being a nursling of the endosperm, it weakens gradually after the onset of endosperm irregularities. It dies only after having used up the nuclear tissue which remains in the seed at the time of the breakdown of the endosperm.

The increased initial rate of growth of both the endosperm and the embryo over that of the corresponding structures of the normal is, possibly, a manifestation of hybrid vigor. It probably enhances the later growth requirements of a hybrid seed over that of a seed which has made a slower start. Such a seed condition will act as an adjuvant in precipitating the events leading to seed collapse.

No explanation can be suggested for the occurrence in the endosperm of hybrid seeds of the micropylar pocket canal, and of the globules released into the central lumen. The frequent association of these globules with nuclei at their point of origin may be of significance for an understanding of this phenomenon.

SUMMARY

Investigations were undertaken of post-syngamic seed failure in a wide cross of Hordeae. Seed development is initiated when *Elymus virginicus* L. is pollinated by *Agropyron repens* (L.) Beauv., but viable seeds are not produced. The development of the *E. virginicus* × *A. repens* seed, relative to that of *E. virginicus* is characterized by the following differences. Double fertilization takes place earlier. The initial rates of growth of the endosperm and the embryo are higher. Some nuclei of the antipodals which are quiescent from the time of pollination until 18 hours thereafter then show signs of activity.

Their chromatin condenses into chromosomes. In certain nuclei the chromosomes are polytenic, showing that endomitosis is taking place or has occurred previously. The endosperm development is regular from the time of its formation until 48 hours after pollination but then becomes irregular; abnormal endosperm nuclei, differing in size, shape, number of nucleoli or in staining reaction to the Feulgen reagent, appear near the antipodals and later elsewhere in the endosperm. These irregular endosperm nuclei are not distributed at random throughout the endosperm but are clustered in the mid-sections of the seed. Parts of the endosperm cytoplasm, correlated in their distribution with that of the irregular nuclei, stain very intensely with pyronin at 36 hours and thereafter. Approximately 12 hours later the endosperm becomes disorganized and eventually the walls of the seed collapse. The embryo associated with an irregular endosperm appears healthy at first but later degenerates and dies with or without undergoing differentiation.

The sequence of events leading to death of the hybrid seed may be summarized as follows. The sperm of *A. repens* is the primary cause of the breakdown. The physiology of the antipodals is disturbed by a stimulus imparted by this sperm in its association with the polar nuclei, as shown by the signs of unusual activity of the antipodal nuclei. The modified antipodals, in their secretory role, alter the nutrient supply to the endosperm. Growth of the latter tissue in turn is impaired by the changed food supply. Mitosis in the endosperm becomes disorderly, resulting in irreversible nuclear changes. Disorganization and breakdown of the endosperm follow, and the tissue disappears from the seed. The embryo, being a nursling of the endosperm, dies by starvation.

ACKNOWLEDGMENTS

The author wishes to express his deep indebtedness to PROFESSORS R. A. BRINK and D. C. COOPER for suggesting this problem and for their interest and encouragement throughout the course of investigations. He also wishes to thank DR. HANS RIS for valuable suggestions concerning the manuscript, and DR. ETLAR L. NIELSEN for assistance in obtaining plants which were used in this study. He is indebted to ALFRED OWCZARZAK for making the photomicrographs.

LITERATURE CITED

BOYES, J. W., and W. P. THOMPSON, 1937 The development of the endosperm and embryo in reciprocal interspecific crosses in cereals. J. Genet. **34**: 203–227.

BRACHET, J., 1940 La détection histochimique des acides pentosenucléiques. C. R. Soc. Biol. **133**: 88–90.

1942 La localisation des acides pentosenucléiques dans les tissus animaux et les oeufs d'amphibiens en voie de développement. Arch. Biol. **53**: 207–257.

BRINK, R. A., and D. C. COOPER, 1940 Double fertilization and development of the seed in angiosperms. Bot. Gaz. **102**: 1–25.

1944 The antipodals in relation to abnormal endosperm behavior in *Hordeum jubatum* × *Secale cereale* hybrid seeds. Genetics **29**: 391–406.

1947 The endosperm in seed development. Bot. Rev., **13**: 423–541.

BRINK, R. A., D. C. COOPER, and L. E. AUSHERMAN, 1944 A hybrid between *Hordeum jubatum* and *Secale cereale* reared from an artificially cultivated embryo. J. Hered. **35:** 66–75.

COOPER, D. C., and R. A. BRINK, 1940 Somatoplastic sterility as a cause of seed failure after interspecific hybridization. Genetics **25:** 593–617.

1944 Collapse of the seed following the mating of *Hordeum jubatum* × *Secale cereale*. Genetics **29:** 370–390.

1945 Seed collapse following the matings between diploid and tetraploid races of *Lycopersicon pimpinellifolium*. Genetics **30:** 376–401.

JOHANSEN, D. A., 1940 Plant Microtechnique. xi + 523 pp. New York: McGraw-Hill, Inc.

KIHARA, H., and I. NISHIYAMA, 1932 Different compatibility in reciprocal crosses of *Avena*, with special reference to tetraploid hybrids between hexaploid and diploid species. Jap. J. Bot. **6:** 245–305.

LA COUR, L. F., 1947 Improvements in plant cytological technique. II. Bot. Rev. **13:** 216–240.

PAINTER, T. S., 1943 Cell growth and nucleic acids in the pollen of *Rhoeo discolor*. Bot. Gaz. **105:** 58–68.

SCHNARF, K., 1929 Embryologie der Angiospermen. xi + 689 pp. Berlin: Gebrüder Borntraeger.

1931 Vergleichende Embryologie der Angiospermen. vii + 354 pp. Berlin: Gebrüder Borntraeger.

STOWELL, R. E., 1945 Feulgen reaction for thymonucleic acid. Stain Techn. **20:** 45–58.

THOMPSON, W. P., and D. JOHNSTON, 1945 The cause of incompatibility between barley and rye. Canad. J. Res. **23:** 1–15.

WAKAKUWA, S., 1934 Embryological studies on the different seed-development in reciprocal interspecific crosses of wheat. Jap. J. Bot. **7:** 151–185.

WATKINS, A. E., 1927 Genetic and cytological studies in wheat. III. J. Genet. **18:** 375–396.

WITKUS, E. R., 1945 Endomitotic tapetal cell divisions in *Spinacia*. Amer. J. Bot. **32:** 326–330.

PLATES I–III

PLATE I

FIGURE 1.—*Elymus virginicus*. Longitudinal section of an ovary showing a mature megagametophyte. × 53.

FIGURE 2.—*E. virginicus*. 60 hours. Remnants of the antipodals between the nucellus (left) and the endosperm (right). × 106.

FIGURE 3.—*E. virginicus*. 60 hours. Feulgen positive threads originating from the nuclei of the endosperm in the micropylar pocket. × 213.

FIGURE 4.—*E. virginicus* × *A. repens*. 48 hours. Large irregular endosperm nucleus located near the antipodals. × 750.

FIGURE 5.—*E. virginicus* × *A. repens*. 120 hours. Irregular endosperm nuclei showing a large number of nucleoli. × 333.

FIGURE 6.—*E. virginicus* × *A. repens*. 48 hours. Dumbbell-shaped endosperm nucleus. The nucleus of the antipodal in the upper right-hand corner contains condensed chromatin. × 213.

FIGURE 7.—*E. virginicus* × *A. repens*. Endosperm nuclei staining differentially with the Feulgen reagent. The nucleus in the upper right-hand corner exhibits a normal staining reaction. × 213.

FIGURE 8.—*E. virginicus* × *A. repens*. 60 hours. Micropylar pocket stained with methyl green–pyronin. The cytoplasm stains much more intensely with pyronin than in the corresponding and comparable cytoplasm of figure 9. A canal is present in the micropylar pocket and a globule bulges out into the central lumen of the endosperm, opposite a nucleus undergoing mitosis. × 213.

FIGURE 9.—*E. virginicus*. 60 hours. Longitudinal section of a normal seed stained with methyl green–pyronin showing that the cytoplasm of the micropylar pocket does not stain as intensely with pyronin as that of the crossed seed shown in figure 8. The sections of figure 8 and figure 9 were stained simultaneously. The dark staining bodies above the embryo are methyl green-stained elongated nuclei (comparable to those of figure 3) which are commonly encountered around the embryo in 60-hour and older normal seeds. × 213.

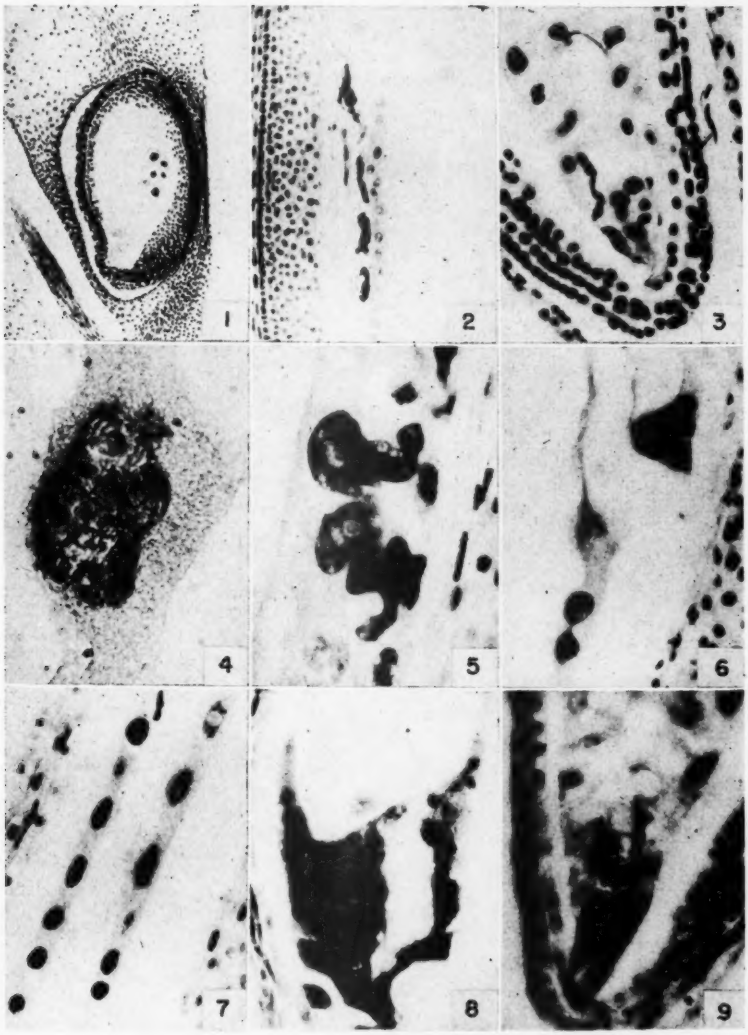


PLATE I

PLATE II

FIGURE 10.—*E. virginicus* × *A. repens*. 72 hours. Micropylar pocket of the endosperm showing the globules which are produced by the endosperm cytoplasm. Stained with methyl green–pyronin. ×213.

FIGURE 11.—*E. virginicus* × *A. repens*. 120 hours. Flattened remnants of the endosperm between the collapsed walls of the seed. × 213.

FIGURE 12.—*E. virginicus* × *A. repens*. 120 hours. Embryo in a seed from which the endosperm has disappeared almost completely. Some nuclei are still dividing. × 213.

FIGURE 13.—*E. virginicus* × *A. repens*. 18 hours. Polar view (lower right) and side view (upper right) of metaphase in the antipodals. × 750.

FIGURE 14.—*E. virginicus* × *A. repens*. 48 hours. Antipodal nucleus showing at least 8 nucleoli. × 750.

FIGURE 15.—*E. virginicus* × *A. repens*. 36 hours. Antipodal nucleus containing more than a hundred chromosomes and dividing into four somewhat unequal groups. Only three of the groups are in focus. × 750.

FIGURE 16.—*E. virginicus* × *A. repens*. Antipodal nucleus (middle left) united with an endosperm nucleus (middle right). In the upper right-hand corner is a normal-looking endosperm nucleus. × 750.

FIGURE 17.—*E. virginicus* × *A. repens*. 18 hours. Prophase in an antipodal nucleus. Note the polytene structure of the chromosomes. × 750.

FIGURES 18 AND 19.—*E. virginicus* × *A. repens*. 24 hours. Metaphase in the antipodals. The units shown are each made of many chromosomes. × 750.

FIGURE 20.—*Hystrix patula* × *A. repens*. 51 hours. A large number of chromosomes present in an antipodal nucleus. × 750.

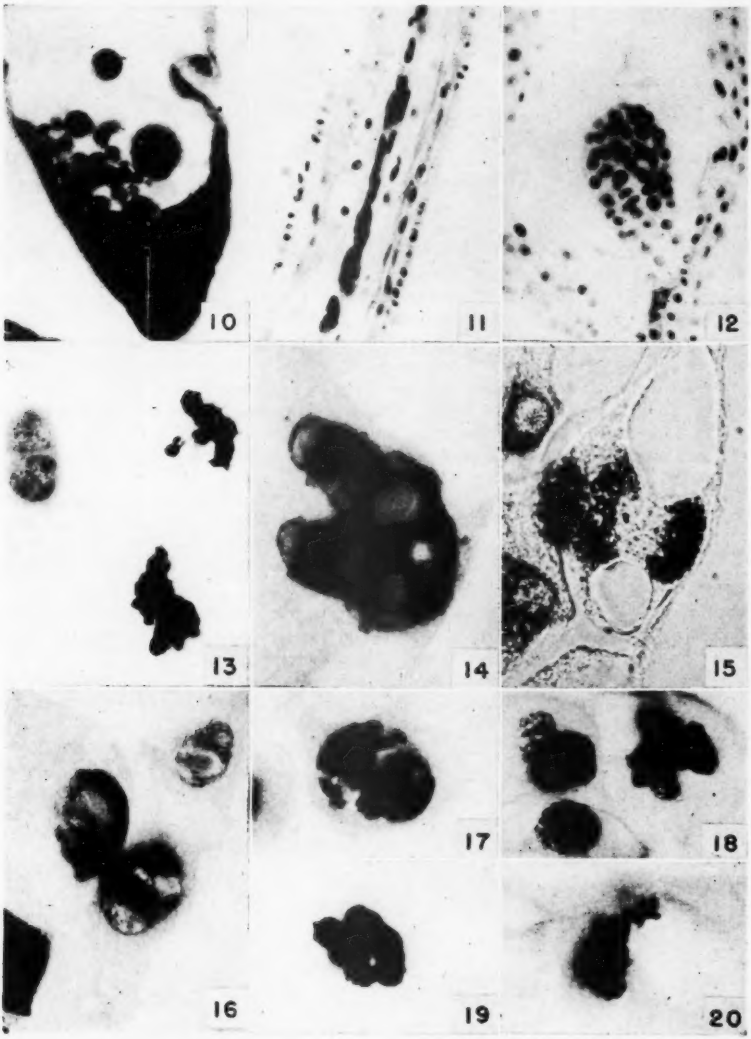


PLATE II

PLATE III

FIGURE 21.—*E. virginicus*. Longitudinal section of an ovary showing a mature megagametophyte. Drawing made from the section shown in figure 1. × 54.

FIGURE 22.—*E. virginicus* × *A. repens*. 48 hours. Dumbbell-shaped endosperm nucleus. Drawing made from the section shown in figure 6. × 236.

FIGURE 23.—*E. virginicus* × *A. repens*. 72 hours. Micropylar pocket of the endosperm showing the globules which are produced by the endosperm cytoplasm. Drawing made from the section shown in figure 10. × 236.

FIGURE 24.—*E. virginicus* × *A. repens*. 60 hours. Micropylar pocket stained with methyl green–pyronin. The cytoplasm stains much more intensely than in the corresponding and comparable cytoplasm of figure 25. Drawing made from the section shown in figure 8. × 255.

FIGURE 25.—*E. virginicus*. 60 hours. Longitudinal section of a normal seed stained with methyl green–pyronin showing that the cytoplasm of the micropylar pocket does not stain as intensely with pyronin as that of the crossed seed shown in figure 24. The sections of figures 24 and 25 were stained simultaneously. Drawing made from the section shown in figure 9. × 255.

FIGURES 26 and 27.—*E. virginicus* × *A. repens*. 24 hours. Metaphase in the antipodals. The chromosomes shown are those visible in one optical section. Drawing made from the sections shown in figures 18 and 19 respectively. × 937.

FIGURE 28.—*Hystrix patula* × *A. repens*. 51 hours. A large number of chromosomes present in an antipodal nucleus. The chromosomes shown are those visible in one optical section. Drawing made from the section shown in figure 20. × 937.

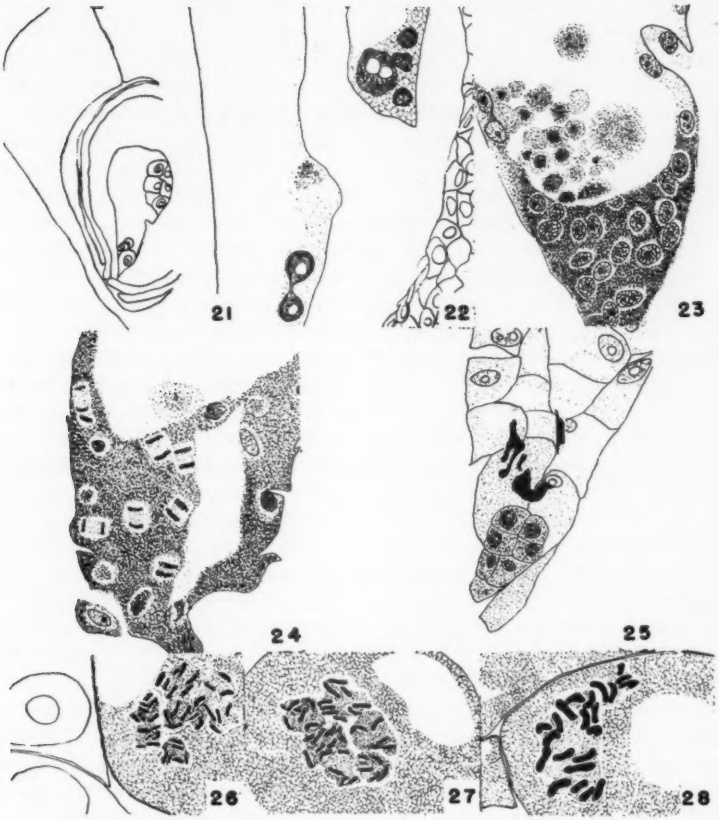


PLATE III

THE B AND C SYSTEMS OF BOVINE BLOOD GROUPS[1]

CLYDE STORMONT,[2] R. D. OWEN[3] AND M. R. IRWIN
University of Wisconsin, Madison, Wisconsin

Received May 29, 1950

INVESTIGATIONS of the antigenic structure of cattle erythrocytes have been in progress in this laboratory during the past decade. In the first two reports of these studies (FERGUSON 1941; FERGUSON, STORMONT and IRWIN 1942), thirty antigens (called A, B, C, E, G, H, I, J, K, M . . . Z, A′, C′ . . . H′) were postulated to explain the serological results. Genetically each of these hypothetical substances, as A, or B or C, etc., appeared as a dominant in contrast to its absence, and no two of them behaved as a single pair of alternatives. From these observations, it seemed probable at the time that each of these 30 blood factors represented a discrete antigen controlled by a single gene. Since the possibility was not overlooked that any one of these antigens might be the product of two or more closely linked genes, a minimum of 30 loci was implied. There were, however, several observations that indicated at least one alternative genetic and serological explanation. For example, FERGUSON (1941) had pointed out that the antigen called E had been noted only in combination with C, but that C occurred in the absence of E. Similarly, antigen K had been observed only in combination with both of the antigens B and G, in a so-called complex "BGK," while B and G had occurred both singly and together in the absence of K (STORMONT, IRWIN and OWEN 1945; also see STORMONT 1950). These and other heretofore unpublished results suggested that each block or complex of associated antigens, like CE or BGK, might be controlled by a single gene, rather than by a system in which each serological characteristic had a separate controlling gene.

Subsequently, the blood factors called F, I′, J′, K′, L′ and Z′ were added to the previous list of 30 (STORMONT 1950). Evidence was also presented for additional associations among bovine blood factors paralleling that, for example, already noted for the factors C and E. These rather simple associations, following the same pattern, were designated as serological subtypes of single blood factors, and for the first time in these studies it was pointed out

[1] From the Departments of Genetics (No. 418), Dairy Husbandry and Veterinary Science, College of Agriculture, UNIVERSITY OF WISCONSIN, in cooperation with the Bureau of Animal Industry, U. S. DEPARTMENT OF AGRICULTURE. This project has been aided by grants from the Research Committee of the Graduate School, University of Wisconsin, from funds supplied by the WISCONSIN RESEARCH FOUNDATION. It was also aided by grants from the HOLSTEIN-FRIESIAN ASSOCIATION OF AMERICA and the AMERICAN GUERNSEY CATTLE CLUB.

[2] Present address: School of Veterinary Medicine, University of California, Davis, California.

[3] Present address: Division of Biology, California Institute of Technology, Pasadena, California.

that the various non-random associations of antigenic factors could be accounted for on a serological basis alone. To be specific, it is a demonstrated immunological fact that single antigenic substances are capable of invoking, and reacting with, a multiplicity of antibodies (see numerous examples and references in LANDSTEINER 1945; KABAT and MAYER 1948). It is also a repeatedly demonstrated fact that antibodies are not absolute in their specificities, but that they are often capable of cross reacting, usually showing graded affinities, with substances closely related to the homologous antigens. Hence, with these non-random associations of bovine blood factors as the initial clue, the explanation was advanced that many of these blood factors are likely the partial serological properties of broader groups of related antigens. The results of numerous absorptions, clearly in agreement with this sort of an explanation, could hardly be explained on any other known serological basis. Now the present report, the first in a series on genetic systems of blood groups in cattle, extends the observations on the original B-G-K and C-E relationships. It is shown that there are numerous blood groups within each of these systems, now called the B and C systems, and that these B and C blood groups involve the majority of the currently recognized bovine blood factors. While the blood groups within each of these systems appear to be these broader groups of serologically related substances and are clearly inherited as if controlled by multiple alleles, various alternative explanations of their nature and possible genetic control are discussed.

MATERIALS AND METHODS

Each of the bovine blood factors, as A or B or C, etc., is recognized by the reaction (hemolysis) of the erythrocytes produced by the combined action of the antibodies in a particular reagent and complement (fresh rabbit serum). For example, erythrocytes possessing a serologically reactive substance capable of combining with the antibodies in the A reagent are hemolysed by the interaction of the antibodies and complement. It is relatively certain, in the presence of adequate controls, that such bloods possess a substance (or substances) similar to or identical with that of the blood cells of the animal whose blood was used as antigen in the production of the A antibodies.

The technics of preparing reagents from antisera produced in rabbits and in cattle against the blood cells of individual cattle, as well as the methods of performing the blood-typing tests, have been described in the papers cited above. Briefly, the tests are carried out as follows: to each of a series of tubes, each containing 0.10 ml of a particular reagent (as A, B, C, etc.) is added 0.05 ml of a two to three percent suspension of the washed erythrocytes of the animal whose cells are to be tested. The tubes are shaken, and to each is then added 0.05 ml of fresh rabbit serum (complement), usually diluted 1 : 2 or used undiluted, to bring the final volume in each tube to approximately 0.20 ml. The mixture of cells, reagent and complement is shaken, and the first reading of the test is made approximately two hours later. The tubes are then shaken and read again after another interval of one and one-half to two hours.

Further readings may be indicated for some of the weakly reactive reagents; usually, however, the second reading is considered final. All tests are performed at room temperature ($26° \pm 4°$). Two operators alternate in making the readings.

The results of the tests are recorded as degrees of hemolysis of the cells, ranging from 0 (no visible hemolysis) to 4 (complete hemolysis). The interpretation of the reactions is to some extent dependent upon familiarity with the individual reagents, and with the variations in their strength over a period of time and in different preparations of the same reagent. Particular attention is given to the time-course of the reactions and to the degrees of hemolysis. Certain of the reagents hemolyze the reactive cells rapidly and completely. With such reagents, a second reading of the tests serves mainly to check the accuracy of the first recordings. Weak, delayed reactions with such reagents are regarded as highly suspicious since in most instances they have been found to be the consequence of negligible cross reactions or of a slight lability of the cells in the complement control. The latter is particularly true of the cells of new-born calves. At the other extreme are the weakly reactive reagents that rarely cause more than delayed, partial hemolysis of the reactive cells. This is true, for example, of the reagent for factor H, even when the antibodies are known to be present in considerable excess. Between these two categories of reagents are those that exhibit considerable variation in the time of onset and the degree of hemolysis produced with different reactive bloods. It is largely the reagents within this category that are divisible by absorption into one or more fractions of antibodies, each fraction often too weak to be of further use in blood typing. The reagents used in serological subtyping (STORMONT 1950) represent such fractions. The latter reagents would appear to be fractions split from a single population of antibodies rather than from mixtures of antibodies sharply different in their specificities.

The chief source of error in these tests is the inadvertent use of faulty preparations of certain of the reagents with the consequence that so-called " false negatives " are obtained. The inclusion of positive controls in the tests is no guarantee that other less pronounced reactions will not be missed. Many of the methods for handling and storing the reagents, as well as the development of the genetic criteria for evaluating the so-called false negatives (referred to elsewhere in this report), evolved over the years during which this study was in progress.

SEROLOGICAL SUBTYPES

The so-called serological subtypes are differentiated by means of antibodies seemingly derived from single populations. For example, two subtypes called T_1 and T_2 of the blood factor T are recognized (STORMONT 1950). Bloods of both of these subtypes react with the reagent called T_2, while only those of subtype T_1 react with the reagent for T_1. Bloods of subtype T_2 only partially exhaust the antibodies in the T_2 reagent. The remaining antibodies, called the T_1 fraction or reagent, react with bloods of subtype T_1 but not T_2. (These

relationships are somewhat analogous to those of the subtypes A_1 and A_2 of the A factor of man; see references in WIENER 1943.) Human bloods of both of the subtypes A_1 and A_2 react with *alpha* antibodies (obtained from group B individuals), but only those of subtype A_1 react with *alpha*$_1$ antibodies prepared by partial absorption of group B serum (from selected individuals) with blood of subtype A_2. (There is disagreement among workers as to whether the A subtypes of man are qualitatively or quantitatively different, since an excess of A_2 cells will absorb all of the *alpha* antibodies.) Since bloods of individual cattle that possess both of the subtypes T_1 and T_2 give the same reactions with the T subtyping reagents as do those with the T_1 factor alone, the former bloods can be differentiated from the latter only by means of genetic tests.

In addition to the subtypes of factor T, two analogous subtypes of each of the factors U and X, and three subtypes of each of the factors O and E′ are recognized. It was previously proposed that the symbols C_1 and C_2 be used to designate the blood types CE and C, respectively, since the serological relationship between CE and C parallels that described for T_1 and T_2. Likewise, the blood types formerly designated YC′ and Y are now designated Y_1 and Y_2, respectively.

It is emphasized again that numerous additional reagents in which the population of antibodies comprising the particular reagents is known to be composed of fractions separable by absorption (largely unpublished observations) were used in this study. As already pointed out, the fractions resulting from partial absorption of the antibodies have been generally too weak to be of practical use in differentiating the potential serological subtypes of the factors involved. On the other hand, the results have indicated that such reagents are reactive with a spectrum or family of closely related substances, hence, the special connotation of the term " blood factor " as defined below.

TERMINOLOGY

The terms antigen and antibody in immunological literature have acquired meanings over a period of years which are generally accepted, although their shortcomings are realized. According to TOPLEY (1933) " an antigen is any substance that, when introduced parenterally into the animal tissues, stimulates the production of an antibody, and, when mixed with that antibody, reacts with it in some observable way." In this broad sense, the erythrocyte is the antigen of these studies. The general definitions of these two terms usually imply a one-to-one correspondence between antigens and antibodies, and, therefore, do not include the possibilities of cross reactions or that more than one kind of antibodies may be engendered against a single antigenic substance. To avoid confusion which might ensue from the general use of the term antigen, the following terminology will be employed in this paper:

1. *Blood factor,* or antigenic factor. The various properties of cattle erythrocytes that are characterized by their reactions with the reagents used in this study are referred to as blood factors (that is, the factors A, B, C_1, C_2,

F, G, etc.). This term does not inquire into the specificity of the reactions, nor does it limit, by definition alone, the range of reaction of the antibodies of the particular reagents. The same blood factor may be common to a number of related antigens, or, in some cases, it may be restricted to a single serologically reactive substance (cf. LANDSTEINER 1945).

2. *Blood group.* The term blood group, as here used, is an inherited characteristic, and may consist of one or more blood factors, or, in certain cases, may be characterized by the absence of a particular reaction or reactions. A parallel example of the latter case is the normal blood group O of man, which is routinely characterized by the failure of the bloods to react with either of the reagents *alpha* (anti-A) or *beta* (anti-B). The terms antigen, antigenic group and blood group might be used synonymously.

3. *Blood group system.* The blood groups that appear to be controlled by the alleles of a single gene are referred to as the groups of a particular system. For example, the letter B is used as the base letter for the groups as well as for the proposed controlling alleles of the B system of bovine blood groups. The B groups are designated as B_b, B_G, B_{BO_1}, $B_{GY_2E'_1}$, $B_{1E'_1}$, etc. The respective alleles are designated B^b, B^G, B^{BO_1}, etc., with the blood factors that characterize the groups written as superscripts. Bloods of the group B_b (representative of one or more potentially reactive groups) are not reactive with any of the reagents of the B system used in this study.

4. *Blood type* or antigenic formula. The blood type of an animal comprises the known blood factors of his erythrocytes as determined by tests with all the available reagents, or, in particular cases, with only those reagents under discussion. For example, the blood type $BFO_2X_1ZA'E'_3J'K'$ is that of one of the bulls of this study, and the factors indicate that his blood cells reacted with the reagents B, F, O_2, O_3, X_1, X_2, Z, A′, E′$_3$, J′ and K′.

EXPERIMENTAL

Materials for the genetic studies consisted of blood samples from 1000 offspring (600 Holstein-Friesians, 300 Guernseys, 50 Jerseys and 50 Brown Swiss), of samples from all but a few of the dams of these individuals and of samples from the 50 sires involved (21 Holstein-Friesians, 20 Guernseys, 4 Jerseys and 5 Brown Swiss). Almost one-half of the samples were drawn from animals of the University of Wisconsin herds; the remainder were obtained from animals of the Pabst herd at Oconomowoc, Wisconsin, the Carnation herd of Carnation Farms, Carnation, Washington, the Blakeford herd of Blakeford Farms, Queenstown, Maryland, and others. Although all the sires were registered animals, a few of the dams were non-registered grades.

With but few exceptions, each of these samples was tested with the reagents for factors A, B, C_1, C_2, F, G, H, I, J, K, O_1, O_2, O_3, P, Q, R, S, T_1, T_2, U_1, U_2, V, W, X_1, X_2, Y_1, Y_2, Z, A′, D′, E′$_1$, E′$_2$, E′$_3$, I′, J′, K′, L′ and Z′.

In view of the extensive nature of the tabular material, it is not possible to show the blood types of all the animals involved in this study. The overall results have been the same in each of the 50 sire-families investigated, and data on a few of these families will illustrate the essential features.

THE B SYSTEM OF BOVINE BLOOD GROUPS

The Holstein-Friesian bull H6 (Wisconsin Admiral Burke Lad 697789), 40 of his progeny, and the dams of these offspring comprised sire-family H6 of this study. The blood type of the bull H6 was $BFGO_1SVY_2ZE'_1$. In table 1 are shown the blood factors of H6 as these appeared in the bloods of a selected group of 20 of his progeny. (The selections in this example, as well as most of those to follow, have been made on the basis of the kinds of matings. We have selected data from those matings in which the dams possessed few or none of the blood factors of the sire or of those blood factors under consideration, since in this initial phase of the analyses particular attention is paid to those blood factors of the progeny that could have only been inherited from their sires.) In the analysis of the ways in which the five factors B, G, O_1, Y_2 and E'_1 of sire H6 appeared in the bloods of his progeny, only two arrays, the combinations BO_1 and $GY_2E'_1$, were demonstrable. The data in table 1 on 20 of his progeny are arranged on the basis of these two arrays. For example, in matings 1–8 the resulting progeny had only the factors B and O_1, while those from matings 11–19 had only the factors G, Y_2 and E'_1. Although the progeny of matings 9, 10 and 20 had all five of these factors, if it be assumed that the two animals from matings 9 and 10 inherited B and O_1 only from the sire and, similarly, the offspring of mating 20 received G, Y_2 and E'_1 from the sire, then the two arrays of blood factors BO_1 and $GY_2E'_1$, as inherited only from the sire, appear as contrasting characters in the cells of these 20 progeny.

TABLE 1

Inheritance of the blood factors B, F, G, O_1, S, V, Y_2, Z *and* E'_1 *of sire H6 (Holstein-Friesian) by 20 of his offspring.*

Mating number	Dam's number	Factors of the sire possessed by the dams	Progeny's number	Factors of the sire in bloods of progeny
1.	289	− − − − − F − − −	742	BO_1 − − − F − − Z
2.	551	− − − − − F − − −	487	BO_1 − − − F S V Z
3.	520	− − − − − F − − Z	M456	BO_1 − − − F − − Z
4.	600	− − − − − F S V −	M463	BO_1 − − − F S − −
5.	620	− − − − − − S V Z	M470	BO_1 − − − − − S V Z
6.	592	BO_1G − − F − V −	M435	BO_1 − − − F − V −
7.	487	BO_1 − − − F − − Z	M450	BO_1 − − − F S V Z
8.	496	BO_1 − − − F − − −	M459	BO_1 − − − F − − −
9	475	− − $GY_2E'_1$F − V Z	M475	$BO_1GY_2E'_1$F S V −
10.	431	$-O_1GY_2E'_1$F − − −	M431	$BO_1GY_2E'_1$F S V −
11.	562	− − − − − F − V Z	716	− − $GY_2E'_1$F − − −
12.	461	− − − − − F − V Z	729	− − $GY_2E'_1$F S − Z
13.	427	$-O_1$ − − − F − − −	739	− − $GY_2E'_1$F S V −
14.	591	− − $-Y_1$ − F S − Z	M413	− − $GY_2E'_1$F S V Z
15.	299	BO_1 − − − F − V −	M441	− − $GY_2E'_1$ − − V −
16.	508	BO_1 − − − F − − Z	715	− − $GY_2E'_1$F − V Z
17.	216	BO_1 − − − F − − Z	M448	− − $GY_2E'_1$F S V Z
18.	366	BO_1G − − F − − Z	M457	− − $GY_2E'_1$F S − Z
19.	606	BO_1G − − F S V Z	M473	− − $GY_2E'_1$F S V Z
20.	594	BO_1G − − F S − −	743	$BO_1GY_2E'_1$F S − Z

There are at least two genetic interpretations of the inheritance of the blood factors B, G, O_1, Y_2 and E'_1 of sire H6 by his 40 progeny. For example, and based in part on the earlier assumption (FERGUSON 1941; FERGUSON, STORMONT and IRWIN 1942) that these blood factors represent discrete genetic characters in themselves each controlled by a single gene non-allelic to the others, it might be assumed that these five factors are controlled by genes at five closely linked loci. (Carrying this sort of an explanation to its logical conclusion, it would be necessary to postulate nine linked loci in all, since each of the subtypes O_1 and E'_1 represents a minimum of three serological characteristics.) Most of the matings listed in table 1, as well as those to follow, are of such a nature that recombinations of the blood factors would have been readily observed had these occurred. In all the data obtained to date, however, the various inherited combinations of the blood factors of this system, as the two arrays BO_1 and $GY_2E'_1$, have behaved as if controlled by single genes, the alleles of an extensive series. While the possibility of closely or absolutely linked genes is not ruled out by the genetic data alone, we have chosen to present the data on the basis of multiple alleles. Other observations previously reported (STORMONT 1950) render the idea of linked genes, as well as the earlier assumption that the blood factors represent discrete antigens in themselves, as a less plausible one.

Now, summarizing the data of table 1 insofar as the five blood factors B, G, O_1, Y_2 and E'_1 are concerned, it would appear that these represent the serological components of distinct blood groups hereinafter referred to as the B groups B_{BO_1} and $B_{GY_2E'_1}$, and these in turn appear to be controlled by alleles designated B^{BO_1} and $B^{GY_2E'_1}$.

The three factors S, V and Z of sire H6 were distributed at random with respect to the B groups B_{BO_1} and $B_{GY_2E'_1}$ in the bloods of his progeny, as is illustrated by the data of table 1. Even the highly frequent factor F is excluded from the B system on the basis of the data from matings 5 and 15. While one of these offspring inherited the group B_{BO_1} and the other $B_{GY_2E'_1}$, both lacked F. We shall omit in each succeeding analysis all those blood factors excluded from the B system in foregoing analyses.

Sixty-six progeny of sire H4 (Miranda Koba Romeo 778235) with the blood type $BFO_2X_1ZA'E'_3J'K'$ and all except four of the dams of these offspring were studied. In a comparison of the ways in which the six factors B, O_2, A', E'_3, J' and K' of H4 appeared in his progeny, only the two arrays $BO_2A'E'_3$ and $O_3J'K'$ were noted. The data on 20 of these offspring, arranged on the basis of these two arrays, are shown in table 2. Assuming that factors O_3, J' and K' of progeny 271H of mating 10 were inherited from the dam, the two arrays $BO_2A'E'_3$ and $O_3J'K'$ are clearly contrasted in matings 1–10 and 11–20, respectively. The subtype factor O_3, not differentiated as such in the presence of O_2 in the blood of sire H4, emerged in his offspring as an alternative to the array of factors $BO_2A'E'_3$. Attention is also called to mating 8 in which offspring D26 had inherited E'_1 from its dam. The factor E'_3 of D26 was inferred from the genetic results alone, since this factor can-

TABLE 2

Inheritance of the blood factors B, O_2, $\underline{O_3}$†, X_1, $\underline{X_2}$†, A′, E'_3, J′ *and* K′ *of sire H4 (Holstein-Friesian) by 20 of his offspring.*

Mating number	Dam's number	Factors of the sire possessed by the dams	Progeny's number	Factors of the sire in bloods of progeny
1.	92	− − − − − − −	174	B O_2 A′ E'_3 − − − X_1
2.	112	− − − − − − −	191	B O_2 A′ E'_3 − − − X_2
3.	112	− − − − − − −	246	B O_2 A′ E'_3 − − − X_2
4.	1503	− − − O_3 J′ K′ −	176	B O_2 A′ E'_3 − − − X_2
5.	F460	− − − O_3 J′ K′ X_2	238	B O_2 A′ E'_3 − − − X_2
6.	D8	− − E'_3 − − − X_2	D30	B O_2 A′ E'_3 − − − X_2
7.	190	− − E'_3 − − − −	384	B O_2 (A′)* E'_3 − − − X_2
8.	D13	− − E'_1 − − − −	D26	B O_2 A′ E'_3† E'_1 − − − X_1
9.	X	(not tested)	182	B O_2 A′ E'_3 X_1
10.	357	− − E'_3 O_3 J′ K′	271H	B O_2 A′ E'_3 O_3 J′ K′ X_2
11.	58	− − − − − − −	183	− − − − − O_3 J′ K′ X_1
12.	127	− − E'_3 − − − −	228	− − − − − O_3 J′ K′ X_1
13.	140	− − E'_3 − − − −	343	− − − − − O_3 J′ K′ X_1
14.	D14	− − E'_3 − − − −	D43	− − − − − O_3 J′ K′ X_2
15.	B4	− − E'_3 O_3 − − −	B13	− − − − − O_3 J′ K′ X_2
16.	96	− − E'_1 − − − −	278	− − − − − O_3 J′ K′ X_2
17.	137	− − E'_1 − − − −	389	− − − − − O_3 J′ K′ X_1
18.	90	B A′ − − − − −	175	− − − − − O_3 J′ K′ X_1
19.	X	(not tested)	210	− − − − − O_3 J′ K′ X_1
20.	258	− − − O_3 J′ K′ −		− − − − − O_3 J′ K′ X_2

*** Blood factors shown within parentheses were either not tested for, or the reaction (a false negative as indicated by the genetic criterion, see text) was believed to have been missed.**

† The underscored serological subtypes were inferred from the genetic results alone, since such factors are not directly detected, serologically, in the presence of a subtype factor inherited from the other parent.

not be recognized in the presence of E'_1 (or E'_2). Likewise, factor X_2 which must have been present along with X_1 in the blood of the sire H4 emerged in the progeny of H4 as the alternative to X_1. On the other hand, the two subtypes of X were distributed at random with respect to the two arrays of the B factors. Summarizing these results, the two B groups $B_{BO_2A'E'_3}$ and $B_{O_3J'K'}$ are recognized, and factor X is excluded from this system.

Prior to the development of reagents for the factors J′ and K′, and those for the subtypes of O and E′, the bloods of 10 offspring of H4 were studied. In these 10 offspring, the groups $B_{BA'}$ and B_b (a group non-reactive with the B reagents developed at that time) appeared as alternatives.

The bull named Colony Ormsby Sir Romeo 781424 headed sire-family H1 of this study. The bloods of 48 offspring of H1 and the dams of all but four of these progeny were tested. The blood type of H1 was $BC_1FGI\text{-}JO_1ST_2X_2Y_2A'$. A comparison of the factors B, C_1, G, I, J, O_1, T_2, Y_2 and A′ as these appeared in the bloods of H1's progeny demonstrated the arrays

TABLE 3

Inheritance of the blood factors B, C_1, G, I, J, O_1, T_2, Y_2 *and* A′ *of sire H1 (Holstein-Friesian) by 20 of his offspring.*

Mating number	Dam's number	Factors of the sire possessed by the dams	Progeny's number	Factors of the sire in bloods of progeny
1.	190	− − − − − − C_1 −	231H	B G I O_1 T_2 A′ − − − − J
2.	A162	− − − − − − − −	A255	B G I O_1 T_2 A′ − − − C_1 J
3.	C	− − O_1 − A′ − − J	C1	B G I O_1 T_2 A′ − − − C_1 −
4.	Z28	− − O_1 T_1 − − C_1 J	Z28C	B G I O_1 T_2 A′ − − − C_1 −
5.	80	− − − − − Y_1 C_1 −	80C	B G I O_1 T_2 A′ − − − C_1 −
6.	A128	− − − − − Y_1 − J	A284	B G I O_1 T_2 A′ − − − C_1 J
7.	10B	− − − − − Y_1 − J	217	B G I O_1 T_2 A′ − − − − J
8.	261	B − − − A′ − C_1 −	70H	B G I O_1 T_2 A′ − − − C_1 −
9.	8B	− G − − − Y_2 C_1 −	215	B G I O_1 T_2 A′ − − − C_1 −
10.	M235	− G − − − Y_2 − −	M282	B G I O_1 T_2 A′ − − − C_1 J
11.	A162	− − − − − − − −	A278	− − − − − − O_1 Y_2 A′ − −
12.	127	− − − − − − − J	9H	− − − − − − O_1 Y_2 (A′)* − −
13.	M185	− − − − − − − J	M281	− − − − − − O_1 Y_2 A′ C_1 −
14.	239	− − − − − − C_1 J	40H	− − − − − − O_1 Y_2 A′ C_1 J
15.	A199	B − O_1 − − − − −	A253	− − − − − − O_1 Y_2 A′ − J
16.	256	B − − − A′ − − −	20H	− − − − − − O_1 Y_2 A′ C_1 −
17.	357	− − − − − − C_1 −	187H	− − − − − − O_1 Y_2 A′ C_1 −
18.	1B	− − − − − − − −	227	− − − − − − O_1 Y_2 A′ − −
19.	49	B − − − A′ − C_1 −	49C	− − − − − − O_1 Y_2 A′ C_1 J
20.	Z15	− − O_1 − A′ − C_1 J	Z15C	− − − − − − O_1 Y_2 A′ C_1 −

*See footnotes of table 2.

$BGIO_1T_2A'$ and O_1Y_2A' (contrasted in matings 1–10 and 11–20, respectively, in the data of table 3) and excluded factors C_1 and J from the B system. The B groups $B_{BGIO_1T_2A'}$ and $B_{O_1Y_2A'}$ are recognized.

In tables 1–3, it has been necessary to refer to the blood types of the dams in only four instances (matings 9, 10 and 20 of table 1, and mating 10 of table 2) to ascertain which of the factors under discussion had been inherited from the sires alone. Although these data have been selected, as pointed out, from those matings in which the dams possessed relatively few or none of the factors under discussion, in most of the matings (including those not shown in the tables) it has not been necessary to know the blood types of the dams in order to determine the B groups of the progeny inherited from their sires. This point is further illustrated in table 3a in which data are shown on an additional 20 offspring of sire H1 and the dams of these progeny. Only in matings 39 and 40 of table 3a is it necessary to refer to the B factors of the dam to determine which of the groups was inherited from the sire H1. It is apparent in these two matings that the full sibs 56H and 206H inherited the group $B_{O_1Y_2A'}$ from their sire and $B_{BGIO_1T_2A'}$ from their dam, since the dam lacked factor Y_2 present in both of the progeny.

The bloods of 52 progeny of sire H5 (Colony Vale Abbekerk Sir Heilo

TABLE 3a

Inheritance of the blood factors B, G, I, O_1, T_2, Y_2 *and* A′ *of sire H1 (also see table 3) by 20 additional offspring.*

Mating number	Dam's number	Factors of the sire possessed by the dams	Progeny's number	B factors of sire in bloods of progeny
21.	M237	−G− − − A′ Y_2	M287	B G I O_1 T_2 A′ − − −
22.	56	−G−O_1 − − −	56C	B G I O_1 T_2 A′ − − −
23.	12	BG− − − A′ Y_2	12C	B G I O_1 T_2 A′ − − −
24.	A207	B− −O_1 − − Y_2	A275	B G I O_1 T_2 A′ − − −
25.	63g	BG− − − − Y_2	32Z	B G I O_1 T_2 A′ − − −
26.	128	BG− − − A′ Y_2	214	B G I O_1 T_2 A′ − − −
27.	23B	−G I − − − −	218	B G I O_1 T_2 A′ − − −
28.	A164	BG− − − A′ Y_2	A269	B G I O_1 T_2 A′ − Y_2 −
29.	A164	BG− − − A′ Y_2	A285	B G I O_1 T_2 A′ − Y_2 −
30.	A236	−G−O_1 − − Y_1	A283	B G I O_1 T_2 A′ − Y_1 −
31.	A144	B− −O_1 − − −	A270	B− − − − − O_1 Y_2 A′
32.	327	B− − − − A′ −	261H	B− − − − − O_1 Y_2 A′
33.	123	−G− − − − Y_2	226	−G− − − − O_1 Y_2 A′
34.	96	−G− − − − Y_2	211	−G− − − − O_1 Y_2 A′
35.	3B	−G I − − − Y_2	209	−G− − − − O_1 Y_2 A′
36.	A123	−G− − − − Y_1	A279	−G− − − − O_1 Y_2 A′
37.	67	−G− − − − Y_1	67C	−G− − − − O_1 $\underline{Y_2}$† A′ Y_1
38.	11ZC	− − − − − A′ Y_1	C11Z	− − − − − − O_1 $\underline{Y_2}$† A′ Y_1
39.	1631	B G I O_1 T_2 A′ −	56H	B G I O_1 T_2 A′ O_1 Y_2 A′
40.	1631	B G I O_1 T_2 A′ −	206H	B G I O_1 T_2 A′ O_1 Y_2 A′

†See footnotes of table 2.

807399) and of the dams of these progeny were tested. The blood type of H5 was $ABC_1FO_1SWY_2D'J'K'$. The data on the inheritance of B factors in a selected group of 20 offspring of H5 (see table 4) demonstrated the B group $B_{BO_1Y_2D'}$ and presented additional evidence for the group $B_{O_3J'K'}$ recognized in the study of family H4, table 2. The factors A and W are excluded as members of the B system by these data of table 4.

Tests were made on the bloods of 23 progeny of the sire H14 (U. of Nebraska Quantor Pan 772753) and on their dams. The blood type of sire H14 was $FGO_3RWX_2Y_1D'J'K'L'$. This formula shows the B factors G, O_3, Y_1, D′, J′ and K′, and the factors F, W and X_2 which were previously excluded from this system. Once again evidence for the B group $B_{O_3J'K'}$ is obtained (data of table 5), but this time as an alternative to the group $B_{GY_1D'}$. The factors R and L′, likewise appearing as alternatives in the progeny of H14, are clearly excluded as members of the B system.

Sire H19 (St. Croixco Hartog Burke 762509) with the blood type $AC_1FGSX_1Y_2E'_1L'$ had 32 offspring whose bloods were tested. The formula of H19 shows only the three B factors G, Y_2 and E'_1, the remaining six factors having been excluded as members of this system in the previous analyses. As might have been expected on the basis of previous information

TABLE 4

Inheritance of the blood factors A, B, O_1, $\underline{O_3}$†, W, Y_2, D′, J′ *and* K′ *of sire H5 (Holstein-Friesian) by 20 of his offspring.*

Mating number	Dam's number	Factors of the sire possessed by the dams	Progeny's number	Factors of sire in bloods of progeny
1.	149	− − − − − − −	340	B O_1 Y_2 D′ − − − A −
2.	63	− − − − − − − W	359	B O_1 Y_2 D′ − − − − W
3.	66	− − − − − − −	66C	B O_1 Y_2 D′ − − − − −
4.	52	− − − O_3 J′ K′ A W	52C	B O_1 Y_2 D′ − − − A W
5.	243	− − − O_3 J′ K′ A W	368	B O_1 Y_2 D′ − − − A −
6.	1866	− Y_1 − O_3 J′ K′ − W	353	Y_1 B O_1 $\underline{Y_2}$† D′ − − − A W
7.	327	B − − − − − − A W	182H	B O_1 Y_2 D′ − − − A −
8.	182	B − − − − − − −	325	B O_1 Y_2 D′ − − − − W
9.	396	− − − O_3 J′ K′ − −	221H	B O_1 Y_2 D′ $\underline{O_3}$† J′ K′ − W
10.	357	− − − O_3 J′ K′ A W	114H	B O_1 Y_2 D′ $\underline{O_3}$† J′ K′ A −
11.	D14	− Y_1 − − − − A −	D44	− − − − O_3 J′ K′ A −
12.	11g-c	− − D′ − − − − −	C11g	− − − − O_3 J′ K′ − W
13.	84H	B − − − − − − A W	224H	− − − − O_3 J′ K′ − W
14.	1732	− Y_2 − O_1 − − A W	298	− − − − O_3 J′ K′ A W
15.	1438	− Y_2 − O_1 − − A W	259	− − − − O_3 J′ K′ A −
16.	B10	− − − O_1 − − A −	B19	− − − − O_3 J′ K′ A W
17.	P3	− − − O_3 J′ K′ A W	P11	− − − − O_3 J′ K′ A −
18.	1745	− − − (O_3) J′ K^b − W	311	− − − − O_3 J′ K′ − W
19.	1469	− − − O_1 J′ K′ A W	296	− − − − O_3 J′ K′ A −
20.	1503	− − − O_3 J′ K′ A W	292	− − − − O_3 J′ K′ A W

†See footnotes of table 2.

on these three B factors (sire-family H6, table 1), they appeared only as the serological constituents of the group $B_{GY_2E'_1}$ in approximately one-half of the progeny of H19 (see table 6), but this time as an alternative to a group (B_b) which is non-reactive with any of the available reagents used in the recognition of these B groups or factors of this system. Attention is called to a similar situation, already mentioned, in which the group $B_{BA'}$ was contrasted with the absence of these two factors in 10 progeny of H4 that were studied prior to the development of some of the more recently described reagents. It is possible that the group (or groups) B_b may be characterized with certain B antibodies yet to be obtained and identified.

Attention is called to matings 19 and 20 of table 6, in which it was not possible to establish which of the two B groups of sire H19 were inherited by the progeny of these two matings. Both of the progeny as well as their parents possessed the group $B_{GY_2E'_1}$. There were parallel situations encountered in various families of this study but these are not shown in any of the preceding tables. For example sire H6 (table 1) heterozygous for the *B* alleles $B^{GY_2E'_1}/B^{BO_1}$ mated two dams presumably of the same genotype to produce offspring of the same genotype. These matings will be called "indeterminate." Of the 1000 offspring studied, there were 39 from indeterminate matings.

TABLE 5

Inheritance of the blood factors G, O_3, R, Y_1, D′, J′, K′ *and* L′ *of sire H14 (Holstein-Friesian) by 20 of his offspring.*

Mating number	Dam's number	Factors of the sire possessed by the dams	Progeny's number	Factors of sire in bloods of progeny
1.	143	− − − − − − − −	760	G Y_1 D′ − − − R −
2.	245	− − − − − − − −	743	G Y_1 D′ − − − R −
3.	410	G O_1 − − − − − − L′	702	G Y_1 D′ O_1 − − − L′
4.	212	G O_1 − − J′ K′ − −	716	G Y_1 D′ O_1 − − − L′
5.	9D	G O_1 − − − − R L′	9	G Y_1 D′ O_1 − − R L′
6.	12D	G O_1 − − − − − L′	12	G Y_1 D′ O_1 − − − L′
7.	410	G O_1 − − − − − L′	702	G Y_1 D′ O_1 − − − L′
8.	489	G O_3 − − J′ K′ − −	778	G Y_1 D′ − − − R −
9.	125	G O_1 − − − − − −	517	G Y_1 D′ − − − − L′
10.	1D	G − − − − − − −	1	G Y_1 D′ − − − R −
11.	244	− − − − − − − −	690	− − − O_3 J′ K′ R −
12.	4D	− O_1 − − − − − L′	4	O_1 − − − $\underline{O_3}$† J′ K′ − L′
13.	6D	G O_1 − − − − − −	6	O_1 − − − $\underline{O_3}$† J′ K′ − L′
14.	2D	− O_1 − − − − − −	2	O_1 − − − $\underline{O_3}$† J′ K′ R −
15.	3D	G O_1 − − − − − −	3	O_1 − − − $\underline{O_3}$† J′ K′ − L′
16.	11D	G O_2 Y_1 D − − − −	11	O_2 − − − $\underline{O_3}$† J′ K′ − L′
17.	13D	G O_1 − − J′ (K′) − −	13	− − − O_3 J′ K′ R −
18.	8D	G O_1 − − − − − −	8	O_1 G − − $\underline{O_3}$† J′ K′ − L′
19.	397	G − − − − − − −	692	G − − O_3 J′ K′ − L′
20.	338	− O_3 − − J′ K′ − L′	703	− − − O_3 J′ K′ R −

†See footnotes of table 2.

The bloods of 58 progeny of the Guernsey bull G5 (Blakeford Lord Jim 256253) with the blood type ABFGKSWE′$_2$ were studied. As noted in his formula, G5 possessed factor K which, as we have previously reported (*loc. cit.*), has been invariably associated with both of the factors B and G. Data on the inheritance of the B factors B, G, K and E′$_2$ of sire G5 in the bloods of 20 of his 58 offspring are shown in table 7. The two arrays BGKE′$_2$ and G are noted, presenting evidence for the B groups $B_{BGKE'_2}$ and B_G. Just as the group B_b emerged as an alternative to the group $B_{GY_2E'_1}$ in the progeny of sire H19 (table 6), the group B_G, a genetic subtype of the group $B_{BGKE'_2}$, appeared in the progeny of G5.

In the foregoing, somewhat detailed presentation, most of the essential features of the transmission of B blood groups by the male parents of this study have been encountered. We shall omit further details, and at this point abstract our data on the segregation of *B* alleles by 47 of the sires that proved to be heterozygous. This summary is given in table 8. For example, sire H1 transmitted the alleles $B^{BGIO_1T_2A'}/B^{O_1Y_2A'}$. The figures 25 after the one allele and 23 after the other (see sire H1, table 8) represent the numbers of offspring in each of the contrasted classes, and similarly for the remaining 46 sires of table 8. The ratios obtained agree satisfactorily with the expected 1 : 1 ratio. Data on 39 progeny in which it was not possible to determine which

TABLE 6

Inheritance of blood factors G, Y_2 *and* E'_1 *of sire H19 (Holstein-Friesian) by 20 of his offspring.*

Mating number	Dam's number	Factors of the sire possessed by the dams	Progeny's number	Factors of the sire in bloods of progeny
1.	59	— — —	58	$GY_2E'_1$
2.	63	— — —	53	$GY_2E'_1$
3.	56	— — —	49	$GY_2E'_1$
4.	46	— — —	138C	$GY_2E'_1$
5.	51	— — —	40B	$GY_2E'_1$
6.	50	G — —	132	$GY_2E'_1$
7.	52	G — —	51	$GY_2E'_1$
8.	64	— — E'_1	43	$GY_2E'_1$
9.	64	— — E'_1	48	$GY_2E'_1$
10.	47	— — E'_1	45	$GY_2E'_1$
11.	58	— — —	57	— — —
12.	4	— — —	136	— — —
13.	64a	— — —	138a	— — —
14.	64	— — E'_1	56	— — —
15.	46a	G — —	46b	— — —
16.	45	G — —	42	— — —
17.	57	G — —	50	G — —
18.	49	G — —	54	G — —
19.	47a	$GY_2E'_1$	133	$GY_2E'_1$?
20.	49a	$GY_2E'_1$	133b	$GY_2E'_1$?

of the two alleles was transmitted by the particular sire are omitted from table 8. The data in table 8 show that several of the sires were heterozygous for the same alleles (compare, for example, sires H4 and H20, H7 and H8, H9 and H18, G4 and G8, and G12 and G20), and that many of the others had at least one allele in common. In all, 14 different alleles at the *B* locus were transmitted by the 20 Holstein-Friesian sires, 14 by the 18 Guernsey sires, six by the four Jersey sires, and seven by the five Brown Swiss sires. Seven of these alleles, however, were common to two or more of the sires of the different breeds, so that a total of only 34 B groups was recognized in the study of the *B* alleles transmitted by the sires.

Inspection of the B groups transmitted by the 47 heterozygous sires (table 8), shows that the 21 bovine blood factors B, G, I, K, O_1, O_2, O_3, P, Q, T_1, T_2, Y_1, Y_2, A′, D′, E'_1, E'_2, E'_3, I′, J′ and K′ are members of this system. Of the remaining 18 blood factors of the total of 38 included in this study, all but one—the extremely rare factor Z′ which was noted in only two of the dams and one of their three progeny—were excluded as members of the B system.

With the knowledge of these 34 groups of the sires, the next step was to attempt to formulate the probable *B* alleles possessed by the dams, even though the dams studied were represented by an average of less than two

TABLE 7

Inheritance of the blood factors B, G, K *and* E'_2 *of sire G5 (Guernsey) by 20 of his offspring.*

Mating number	Dam's number	Factors of the sire possessed by the dams	Progeny's number	Factors of the sire in bloods of progeny
1.	E4	————	E10	BGKE'_2
2.	E4	————	E13	BGKE'_2
3.	M2	————	M5	BGKE'_2
4.	M2	————	M7	BGKE'_2
5.	C2	—G——	C4	BGKE'_2
6.	N1	—G——	N4	BGKE'_2
7.	I4	—G——	I6	BGKE'_2
8.	A3	—G—E'_1	A4	BGKE'_2†E'_1
9.	I2	BG——	I8	BGKE'_2
10.	D7	BG——	D9	BGKE'_2
11.	E6	————	E12	—G——
12.	E7	————	E9	—G——
13.	M1	———E'_1	M6	—G——
14.	M1	———E'_1	M9	—G——
15.	T1	———E'_1	T5	—G——
16.	E1	———E'_1	E11	—G——
17.	Y3	BGKE'_1	Y6	—G—E'_1
18.	A2	BGKE'_1	A3	—G—E'_1
19.	A2	BGKE'_1	A10	—G—E'_1
20.	J2	BGKE'_1	J11	—G—E'_1

†See footnotes of table 2.

offspring each. There were a number of helpful clues from the data of table 8, such as the peculiarities in the reactive patterns of many of these groups and the noticeable trend of differences among the breeds. For example, a Holstein-Friesian with the comparatively rare subtype factor T_2 might be expected to have the allele $B^{BGIO_1T_{2A'}}$, since this is the only one of the 34 known groups having this factor as a component. The absence of any one or more of the five factors of this group, provided errors in blood typing could be ruled out, would, of course, invalidate this hypothesis. Likewise, an animal with E'_2 might be expected to possess the allele $B^{BGKE'_2}$. Furthermore, there are numerous distinctive combinations of the blood factors effected by the various combinations of the 34 alleles. Thus, an animal with the factors B, G, I, K, O_2, Y_1, A′, E'_1 and I′ might be expected to be heterozygous for the alleles $B^{BGKO_2Y_1A'E'_3I'}/B^{IE'_1}$. In addition, none of the paired combinations of factors B and J′, G and J′, I and K, I and T_1, K and T_1 (or T_2), K and O_1, for example, occur as the common properties of any one of the 34 B groups. Therefore, an animal possessing such pairs of factors would probably be heterozygous.

The application of these considerations is illustrated by the data of tables 9a and 9b. In table 9a the B factors detected in the bloods of 20 dams and

TABLE 8

The segregation of B alleles by 47 bulls with the numbers of offspring in each of the contrasted classes.

	Holstein-Friesians		Guernseys *(continued)*
H1	$B^{BGIO_1T_2A'}(25)/B^{O_1Y_2A'}(23)$	G7	$B^{O_1T_1E'_3}(7)/B^{O_3}(9)$
H2	$B^{BGIO_1T_2A'}(13)/B^{BO_2A'E'_3}(12)$	G8	$B^{BGKO_2Y_1A'E'_3I'}(3)/B^{G}(3)$
H3	$B^{BGIO_1T_2A'}(5)/B^{BO_1Y_2D'}(3)$	G10	$B^{BGKE'_2}(2)/B^{IQE'_1}(5)$
H4	$B^{BO_2A'E'_3}(35)/B^{O_3J'K'}(31)$	G11	$B^{BGKO_2Y_1A'E'_3K'}(9)/B^{O_1I'}(12)$
H5	$B^{BO_1Y_2D'}(26)/B^{O_3J'K'}(24)$	G12	$B^{BGKE'_2}(4)/B^{IE'_1}(1)$
H6	$B^{BO_1}(15)/B^{GY_2E'_1}(23)$	G13	$B^{BGKO_2Y_1A'E'_3K'}(5)/B^{b}(3)$
H7	$B^{BGKE'_2}(14)/B^{GY_2E'_1}(15)$	G14	$B^{IE'_1}(8)/B^{I'}(8)$
H8	$B^{BGKE'_2}(8)/B^{GY_2E'_1}(11)$	G15	$B^{IE'_1}(1)/B^{O_1}(8)$
H9	$B^{BO_2A'E'_3}(14)/B^{E'_3}(13)$	G16	$B^{BGKE'_2}(4)/B^{BI}(9)$
H11	$B^{O_1A'}(31)/B^{O_3J'K'}(23)$	G17	$B^{BGKE'_2}(5)/B^{b}(3)$
H12	$B^{O_1A'}(7)/B^{O_3D'E'_3}(6)$	G18	$B^{BGKO_2Y_1A'E'_3K'}(2)/B^{IE'_1}(8)$
H13	$B^{GY_2E'_1}(3)/B^{O_1A'}(5)$	G19	$B^{IE'_1}(8)/B^{b}(7)$
H14	$B^{GY_1D'}(10)/B^{O_3J'K'}(13)$	G20	$B^{BGKE'_2}(2)/B^{IE'_1}(4)$
H15	$B^{O_3J'K'}(10)/B^{E'_3}(9)$		
H16	$B^{BGKO_3Y_2A'}(17)/B^{O_1Y_2A'}(7)$		Brown Swiss
H17	$B^{BO_1Y_2D'}(6)/B^{O_1A'}(7)$	S1	$B^{BPY_2}(5)/B^{O_1T_1Y_2E'_3}(3)$
H18	$B^{BO_2A'E'_3}(10)/B^{E'_3}(14)$	S2	$B^{BO_3A'D'}(10)/B^{O_3}(9)$
H19	$B^{GY_2E'_1}(19)/B^{b}(13)$	S3	$B^{BPY_2}(2)/B^{O_1}(1)$
H20	$B^{BO_2A'E'_3}(6)/B^{O_3J'K'}(6)$	S4	$B^{BPY_2}(1)/B^{BI}(2)$
H21	$B^{BO_1Y_2D'}(13)/B^{I'}(16)$	S5	$B^{BO_1QT_1}(7)/B^{O_1T_1Y_2E'_3}(8)$

	Guernseys		Jerseys
G1	$B^{BGKO_2Y_1A'E'_3I'}(3)/B^{O_1T_1E'_3I'}(6)$	J1	$B^{GD'}(9)/B^{QD'E'_1}(4)$
G3	$B^{BI}(5)/B^{O_1I'}(14)$	J2	$B^{O_3Y_1D'J'K'}(5)/B^{QD'E'_1}(4)$
G4	$B^{BGKO_2Y_1A'E'_3I'}(12)/B^{G}(14)$	J3	$B^{O_3Y_1D'J'K'}(3)/B^{BO_1I'}(11)$
G5	$B^{BGKE'_2}(23)/B^{G}(27)$	J4	$B^{BGKO_2Y_1A'E'_3K'}(7)/B^{BI}(9)$
G6	$B^{BGKO_2Y_1A'E'_3I'}(5)/B^{O_3}(7)$		

their progeny of sire-family H2 (Colony Vale Sir Romeo 4th 832252) are listed. From table 8, it is seen that H2 was heterozygous for the alleles $B^{BGIO_1T_2A'}/B^{BO_2A'E'_3}$. The data of table 9a are arranged on the basis of the two arrays of the sire's B factors, as they occurred in the progeny. The two arrays $BGIO_1T_2A'$ and $BO_2A'E'_3$ are clearly contrasted in the matings 1–10 and 11–20, respectively. The dams of matings 1, 2 and 12 had only the three factors O_3, J′ and K′ which in Holstein-Friesians have been indicative, invariably, of the group $B_{O_3J'K'}$. Also, the factors O_3, J′ and K′ of the dams of matings 8 and 13, and factors J′ and K′ of the dams of matings 3, 6, 7, 14 and

TABLE 9a

B factors in the bloods of 20 progeny of sire H2 (sire's genotype $B^{BGIO_1T_2A'}/B^{BO_2A'E'_3}$) and the dams of these progeny.

Mating number	Dam's number	B factors in blood of dams	Progeny's number	B factors in blood of progeny
1.	183	———O_3 ———————J′K′	306	BGIO_1T_2—A′————
2.	259	———O_3 ———————J′K′	12H	BGIO_1T_2—A′———J′K′
3.	270	B——O_1 —Y_2—D′——J′K′	174H	BGIO_1T_2—A′——J′K′
4.	135	BG—O_1 —Y_2—D′E'_1———	334	BGI$O_1T_2Y_2$A′D′————
5.	1623	B——O_1 —Y_1—D′E'_3———	H3	BGI$O_1T_2Y_2$A′D′————
6.	288	B——O_2 ——A′—E'_3—J′K′	106H	BGIO_1T_2—A′———J′K′
7.	352	BGIO_1 T_2—A′———J′K′	214H	BGIO_1T_2—A′——J′K′
8.	242	—G—O_3 —Y_2——E'_1—J′K′	371	BGIO_1T_2—A′——J′K′
9.	211	—G—O_1 —Y_2A′—E'_1———	277H	BGI$O_1T_2Y_2$A′—E'_1———
10.	6H	BGIO_1 T_2Y_2A′—E'_1———	218H	BGIO_1T_2—A′————
11.	105	————P———————I′——	335	B——O_2——A′—E'_3———
12.	175	———O_3 ———————J′K′	315	B——O_2——A′—E'_3—J′K′
13.	307	———O_3 —Y_1——E'_3—J′K′	101H	B——O_2——A′—E'_3—J′K′
14.	294	———O_1 ——A′———J′K′	91H	B——O_2——A′—E'_3—J′K′
15.	4H	———— ————E'_3———	219H	B——O_2——A′—E'_3———
16.	D19	—G—— —Y_1——E'_1———	D36	B——O_2—Y_1A′—E'_1———
17.	153	—G—O_1 —Y_2A′————	309	BG—O_1—Y_2A′—E'_3———
18.	1723	B——O_1 —Y_2—D′E'_3I′——	H22	B——O_2——A′—E'_3I′——
19.	191	B——O_2 ——A′—E'_3———	16H	B——O_2——A′—E'_3———
20.	1690	B——O_2 ——A′—E'_3—J′K′	327	B——O_2——A′—E'_3———

20 are indicative of the group $B_{O_3J'K'}$. A fair test of this assumption was the appearance of both of the factors J′ and K′ in eight of the 10 progeny of these matings, and their mutual absence in the remaining progeny. Likewise, the presence of the four factors B, O_1, Y_2 and D′ in animals of this breed is the key to the group $B_{BO_1Y_2D'}$. The dams of matings 3, 4, 5 (factor Y_2 is assumed to be present in this animal but could not be differentiated in the presence of Y_1) and 18, therefore, probably had the group $B_{BO_1Y_2D'}$. The presence of both of the factors Y_2 and D′ in the progeny of matings 4 and 5, as well as the absence of Y_2 and D′ in the offspring of mating 3, and the absence of O_1, Y_2 and D′ in the progeny of mating 18, were in agreement with this assumption. Similarly, the factors G, Y_2 and E'_1 of the dams of matings 4, 8, 9, 10 and 16 (factor Y_2 is assumed but cannot be differentiated in the presence of Y_1) indicated that the group was $B_{GY_2E'_1}$. The absence of factor E'_1 in the offspring of mating 4, of factors Y_2 and E'_1 in the progeny of matings 8 and 10, of G in the progeny of mating 16, and the presence of all three of these factors in the offspring of mating 9 did not disagree with this assumption. Other previously recognized groups indicated by the patterns of

TABLE 9b

The probable B genotypes of the animals of sire-family H2 (sire's genotype $B^{BGIO_1T_2A'}/B^{BO_2A'E'_3}$).

Mating number	Dam's number	Probable B alleles of dams	Progeny's number	Probable B alleles of progeny
1.	183	$B^{O_3J'K'}/B^{b}$	306	$B^{BGIO_1T_2A'}/B^{b}$
2.	259	$B^{O_3J'K'}/B^{b}$	12H	$B^{BGIO_1T_2A'}/B^{O_3J'K'}$
3.	270	$B^{BO_1Y_2D'}/B^{O_3J'K'}$	174H	$B^{BGIO_1T_2A'}/B^{O_3J'K'}$
4.	135	$B^{BO_1Y_2D'}/B^{GY_2E'_1}$	334	$B^{BGIO_1T_2A'}/B^{BO_1Y_2D'}$
5.	1623	$B^{BO_1Y_2D'}/B^{Y_1E'_3}$	H3	$B^{BGIO_1T_2A'}/B^{BO_1Y_2D'}$
6.	288	$B^{BO_2A'E'_3}/B^{O_3J'K'}$	106H	$B^{BGIO_1T_2A'}/B^{O_3J'K'}$
7.	352	$B^{BGIO_1T_2A'}/B^{O_3J'K'}$	214H	$B^{BGIO_1T_2A'}/B^{O_3J'K'}$
8.	242	$B^{GY_2E'_1}/B^{O_3J'K'}$	371	$B^{BGIO_1T_2A'}/B^{O_3J'K'}$
9.	211	$B^{GY_2E'_1}/B^{O_1Y_2A'}$	277H	$B^{BGIO_1T_2A'}/B^{GY_2E'_1}$
10.	6H	$B^{BGIO_1T_2A'}/B^{GY_2E'_1}$	218H	$B^{BGIO_1T_2A'}/B^{BGIO_1T_2A'}$
11.	105	$B^{PI'}/B^{b}$	335	$B^{BO_2A'E'_3}/B^{b}$
12.	175	$B^{O_3J'K'}/B^{O_3J'K'}$	315	$B^{BO_2A'E'_3}/B^{O_3J'K'}$
13.	307	$B^{O_3J'K'}/B^{Y_1E'_3}$	101H	$B^{BO_2A'E'_3}/B^{O_3J'K'}$
14.	294	$B^{O_1A'}/B^{O_3J'K'}$	91H	$B^{BO_2A'E'_3}/B^{O_3J'K'}$
15.	4H	$B^{E'_3}/B^{b}$	219H	$B^{BO_2A'E'_3}/B^{b}$ or $B^{E'_3}$
16.	D19	$B^{GY_2E'_1}/B^{Y_1E'_1}$	D36	$B^{BO_2A'E'_3}/B^{Y_1E'_1}$
17.	153	$B^{GO_1Y_2}/B^{O_1Y_2A'}$	309	$B^{BO_2A'E'_3}/B^{GO_1Y_2}$
18.	1723	$B^{BO_1Y_2D'}/B^{E'_3I'}$	H22	$B^{BO_2A'E'_3}/B^{E'_3I'}$
19.	191	$B^{BO_2A'E'_3}/B^{b}$	16H	$B^{BO_2A'E'_3}/B^{b}$ or $B^{BO_2A'E'_3}$
20.	1690	$B^{BO_2A'E'_3}/B^{O_3J'K'}$	327	$B^{BO_2A'E'_3}/B^{BO_2A'E'_3}$

reaction were the groups $B_{BGIO_1T_2A'}$ in the dams of matings 7 and 10, the group $B_{E'3}$ of dam 4H (mating 15), the group $B_{BO_2A'E'3}$ in dam 288 (mating 6), the group $B_{O_1A'}$ in dam 294 (mating 14), and either the group $B_{O_1Y_2A'}$ or $B_{O_1A'}$ in the dams of matings 9 and 17.

B groups either indicated or definitely proved by these data of table 9a were the groups $B_{PI'}$ of dam 105 (mating 11), $B_{Y_1E'_3}$ of the dams of matings 5 and 13, $B_{Y_1E'_1}$ of dam D19 (mating 16), $B_{GO_1Y_2}$ of dam 153 (mating 17) and $B_{E'3I'}$ of dam 1723 (mating 18). The absence of both of the factors P and I′ in the progeny of mating 11 was proof of the group $B_{PI'}$. The absence of both of the factors Y_1 and E'_3 in the offspring of mating 5, and the absence of Y_1 in the offspring of mating 13 were suggestive of the group $B_{Y_1E'_3}$. The fact that the offspring of dam 153 had inherited the three factors G, O_1 and Y_2 from 153 alone suggested the group $B_{GO_1Y_2}$. The presence of both of the factors Y_1 and E'_1, and the absence of factor G in the progeny of dam D19 indicated the group $B_{Y_1E'_1}$.

Additional evidence for the existence of groups such as $B_{Y_1E'_3}$ and $B_{GO_1Y_2}$ has come from the study of other sire-families. For example, dam 307 (mating 13, table 9a) was the daughter of sire H5 (table 4) known to be heterozygous for the alleles $B^{BO_1Y_2D'}/B^{O_3J'K'}$. Accordingly, 307 must have inherited the group $B_{Y_1E'_3}$ from her dam, and transmitted the allele $B^{O_3J'K'}$ to her offspring (101H, mating 13, table 9a). Dam 153 (mating 17, table 9a) produced two offspring in matings to sire H11 who was known to be heterozygous for $B^{O_1A'}/B^{O_3J'K'}$. Both of these full sibs inherited the group $B_{O_3J'K'}$ from their sire H11, while one inherited the group $B_{GO_1Y_2}$ and the other the group $B_{O_1Y_2A'}$ from their dam 153. Similarly, the proposed groups of many of the dams of table 9a either were tested in other matings or corresponded exactly to expectations according to the *B* alleles possessed by their parents.

The proposed genotypes of the animals whose B factors were listed in the table 9a are shown in table 9b. Except for the progeny of matings 15 and 19, in which it was not possible to determine the alleles that were transmitted by the dams, the information is complete. Further tests of the genotypes of a number of these progeny were also obtained. For example, the offspring of mating 5 was sire H3 (table 8). Likewise, the offspring of mating 18 was sire H22 whose three offspring have not been included as a part of the tabular material of this study, but, nevertheless, added to the tests of the proposed genotype of this bull. The origin of comparatively rare homozygotes is shown by the data of matings 10 and 20 of table 9b.

In table 10 are summarized the B genotypes established in a number of the animals of several closely related sire-families in a large herd of Guernseys. Part I of table 10 shows the proposed genotypes of eight dams and their 10 offspring by sire G4 (Langwater Reveller 206846) heterozygous for the alleles $B^{BGKO_2Y_1A'E'_3I'}/B^{G}$. The information given in the right column of this table indicates that offspring B3 of mating 1 also appeared as the dam in matings 3 and 6 to her own sire. The genotypes of 10 offspring of sire G5 (Blakeford Lord Jim 246817), a son of sire G4, and those of the dams of these 10 offspring are shown in part II of table 10. Although the dam of G5 was not tested, it appears that G5 must have inherited the group $B_{BGKE'_2}$ from his dam and B_G from his sire G4. Sire G10 (Blakeford Steadfast King 299118), son of G5 × A2 (mating 13), headed the sire-family whose genotypes are shown in part III of table 10. In parts IV and V of the table, data are presented on certain of the animals of the respective families of the bull G6 (Langwater Ambassador 216197) and his son G7 (Blakeford Ensign 246817). Attention is also called to several of the maternal parents as, for example, the animal coded B2 which appeared as the dam of the progeny of matings 1, 2, 15, 28 and 35, and the dam E1 of the progeny of matings 8, 20, 29, 33 and 34. The progeny of matings 8, 9 and 10 represented three generations in a single family. Except for five of the animals (the progeny of matings 5, 26, 31 and 35, and the dam of mating 27) the complete B genotype of each of the animals represented was clearly established. The allele B^{G}

TABLE 10

The B alleles segregated by representative animals of one large herd of Guernsey cattle.

Mating number	Dam's number	Probable B alleles of dams	Progeny's number	Probable B alleles of progeny	Reference matings
		I. Matings to sire G4 ($B^{BGKO_2Y_1A'E'_3I'}/B^{G}$)			
1.	B2	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	B3	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	3, 6,
2.	B2	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	B4	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	5,
3.	B3	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	B14	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	...
4.	H1	$B^{I'}/B^{G}$	G8	$B^{BGKO_2Y_1A'E'_3I'}/B^{G}$	Table 8
5.	B4	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	G9	?*/$B^{BGKO_2Y_1A'E'_3I'}$	...
6.	B3	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	B5	$B^{G}/B^{O_1T_1E'_3}$	...
7.	I1	$B^{BI'}/B^{IE'_1}$	I2	$B^{G}/B^{BI'}$	16, 32,
8.	E1	$B^{IQE'_1}/B^{b}$	E2	$B^{G}/B^{IQE'_1}$	9,
9.	E2	$B^{IQE'_1}/B^{G}$	E3	$B^{G}/B^{IQE'_1}$	10,
10.	E3	$B^{IQE'_1}/B^{G}$	E8	$B^{G}/B^{IQE'_1}$	...
		II. Matings to sire G5 ($B^{BGKE'_2}/B^{G}$) son of G4			
11.	E4	B^{O_3}/B^{b}	E10	$B^{BGKE'_2}/B^{b}$	...
12.	I4	$B^{O_1T_1E'_3}/B^{G}$	I6	$B^{BGKE_2}/B^{O_1T_1E'_3}$	...
13.	A2	$B^{BGKE'_2}/B^{IQE'_1}$	G10	$B^{BGKE'_2}/B^{IQE'_1}$	See III
14.	A3	$B^{G}/B^{IQE'_1}$	A4	$B^{BGKE'_2}/B^{IQE'_1}$	...
15.	B2	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	B20	$B^{BGKE'_2}/B^{BGKO_2Y_1A'E'_3I'}$	...
16.	I2	$B^{BI'}/B^{G}$	I11	$B^{G}/B^{BI'}$	...
17.	E6	B^{O_3}/B^{b}	E12	B^{G}/B^{O_3}	...
18.	E7	B^{O_3}/B^{b}	E9	B^{G}/B^{b}	...
19.	A2	$B^{BGKE'_2}/B^{IQE'_1}$	A3	$B^{G}/B^{IQE'_1}$	14,
20.	E1	$B^{IQE'_1}/B^{b}$	E11	B^{G}/B^{b}	...

TABLE 10—(*continued*)

Mating number	Dam's number	Probable B alleles of dams	Progeny's number	Probable B alleles of progeny	Reference matings
		III. Matings to sire G10 ($B^{BGKE'_2}/B^{IQE'_1}$) son of G5 × A2			
21.	06	$B^{G}/B^{IQE'_1}$	09	$B^{BGKE'_2}/B^{G}$	...
22.	03	$B^{BGKO_2Y_1A'E'_3I'}/B^{BGKE'_2}$	04	$B^{BGKE'_2}/B^{BGKE'_2}$	...
23.	A2	$B^{BGKE'_2}/B^{IQE'_1}$	A8	$B^{IQE'_1}/B^{IQE'_1}$	...
24.	H2	$B^{BGKO_2Y_1A'E'_3I'}/B^{G}$	H7	$B^{IQE'_1}/B^{G}$	...
25.	V8	$B^{BGKO_2Y_1A'E'_3I'}/B^{IE'_1}$	V12	$B^{IQE'_1}/B^{IE'_1}$	...
		IV. Matings to sire G6 ($B^{BGKO_2Y_1A'E'_3I'}/B^{O_3}$)			
26.	H1	$B^{I'}/B^{G}$	H4	$B^{BGKO_2Y_1A'E'_3I'}/?$ *	...
27.	L1	$B^{IE'_1}/?$ *	L2	$B^{BGKO_2Y_1A'E'_3I'}/B^{IE'_1}$	...
28.	B2	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	G7	$B^{O_3}/B^{O_1T_1E'_3}$	See V
29.	E1	$B^{IQE'_1}/B^{b}$	E7	B^{O_3}/B^{b}	...
30.	A2	$B^{BGKE'_2}/B^{IQE'_1}$	A7	$B^{O_3}/B^{IQE'_1}$	...
		V. Matings to sire G7 ($B^{O_1T_1E'_3}/B^{O_3}$) son of G6 × B2			
31.	E4	B^{O_3}/B^{b}	E5	$B^{O_1T_1E'_3}/?$ *	...
32.	I2	$B^{BI'}/B^{G}$	I4	$B^{O_1T_1E'_3}/B^{G}$	12,
33.	E1	$B^{IQE'_1}/B^{b}$	E4	B^{O_3}/B^{b}	11, 31,
34.	E1	$B^{IQE'_1}/B^{b}$	E6	B^{O_3}/B^{b}	17,
35.	B2	$B^{BGKO_2Y_1A'E'_3I'}/B^{O_1T_1E'_3}$	B8	$?^{*}/B^{O_1T_1E'_3}$	...

* The alternative allele was not established.

of the bull G8 (mating 4), who was used in a different herd, was established by a study of his progeny. As may be noted (mating 4) each of the groups B_G and $B_{I'}$ possessed by the dam of G8 are genetic subtypes of the group $B_{BGKO_2Y_1A'E'_3I'}$ inherited by G8 from his sire G4. Of the ten groups represented by the alleles of table 10, the four groups $B_{BGKO_2Y_1A'E'_3I'}$, $B_{BGKE'_2}$, $B_{O_1T_1E'_3}$ and $B_{IQE'_1}$ are sufficiently differentiated serologically to be recognized in any of their heterozygous combinations and to be readily traced in the animals of the different generations. These data along with those of tables 9a and 9b are representative of the overall picture of the inheritance of the B blood groups of cattle.

Table 11 is a compilation of all the B groups (a total of 80) or heritable combinations of the 21 B factors recognized in this study, and these are listed for each of the four breeds that were represented. For example, of the 47 groups recognized in Holstein-Friesians, 22 were found in one or more of the three other breeds, as shown in the table. Although precise estimates of the frequencies of the various alleles controlling the B groups have not been obtained, it is apparent from the data of table 15 that the breeds are fairly well differentiated on the basis of these groups. Thus, the groups $B_{GY_2E'_1}$ and $B_{O_3J'K'}$, which were the two most frequently encountered in the parental animals of the Holstein-Friesians, have not been observed in any of the Guernseys nor Jerseys tested, although both of these groups were recognized in Brown Swiss. The groups $B_{BGKO_2Y_1A'E'_3I'}$ and $B_{IE'_1}$, the two most frequently encountered in the parental animals of the Guernsey breed, have not been observed to date in animals of the other three breeds. On the other hand, such groups as $B_{O_1T_1E'_3}$ and $B_{I'}$ were noted in each of the four breeds. It is well to mention that parallel differences have been observed in the frequencies of the different groups in the so-called lines or sub-populations within breeds. The marked differences in the lines pose a major problem in obtaining adequate samples for the estimation of allele-frequencies in any breed.

Considering the total number of B groups, it should be emphasized that there must be many others in addition to the 80 recognized in this study. On the other hand, however, it is our belief that the majority of B groups in the Holstein-Friesian and Guernsey breeds have now been recognized. This opinion is supported by a large number of tests in connection with questionable parentage arising in the registration of pure bred animals, not included in this report.

In the presentation of the genetic results, we have refrained from mentioning previously some of the difficulties encountered in the serological tests. These difficulties were only fully understood following recognition of the various groups of the B system, and some of the peculiarities in their reactions with the characterizing antibodies. For example, we have noted at various places in the tables (as matings 7 of table 2 and 12 of table 3) that certain of the factors of the groups were either not recognized (false-negative reactions) or were not tested for. The genetic observations have shown that a number of the reactions, such as the K′ reaction of those bloods with the

TABLE 11

The genetic groups of B factors recognized in four breeds of dairy cattle.

Holstein-Friesians	Guernseys	Brown Swiss	Jerseys
$BGKO_2Y_1A'E'_3$ *	$BGKO_2Y_1A'E'_2I'$	$BGKE'_2$ #*	$BGKO_2Y_1A'E'_3K'$ #
$BGKO_3A'E'_3$	$BGKO_2Y_1A'E'_3K'$ #	BI##	Y_1A'
$BGKO_3Y_2A'$	$BGKO_2Y_1A'E'_3$*	$BO_2A'D'$	E'_3 **
$BGKY_2A'$ *	$BGKO_3A'K'$	BPY_2	GD'
$BGKE'_2$ #*	$BGKY_2A'$	B***	$QD'E'_1$
$BGKE'_3$ *	$BGKE'_2$ #*	BO_1QT_1	I'*#*
$BO_2Y_1A'E'_3$	$BGKE'_3$ *	$GO_1E'_1$	BI'*#
$BO_2A'E'_3$	BGO_1Y_1	$GY_2E'_1$ ***‡	BO_1I'
BO_1*	$BGIY_1$	$O_1T_1E'_3$*#*	$O_1T_1E'_3$*#*
BO_1Y_2D'	BO_1*	$O_1T_1E'_2I'K'$ ##	$T_1E'_3$
BO_1O	BI##	$O_1T_1Y_2E'_3$	$O_3Y_1D'J'K'$
BO_1D'	GI*	O_1#*	
B***	I*	O_1I' ##	
$BGIO_1T_2A'$	IE'_1	I'*#*	
G*	IOE'_1	QI'	
GI*	G*	E'_1 ***	
I*	GE'_3	$Y_2A'D'E'_1$	
GO_1	GO_3	$O_2E'_3$	
GO_1Y_2	$GO_3E'_3$	O_3#*	
$GY_2E'_1$ ***	O_1#*	$O_3J'K'$ ***	
GY_1D'	O_1Y_2A' *	b#*	
Y_1D'*	$O_1T_1E'_3$*#*		
$Y_1A'E'_3$	$O_1T_1E'_2I'$		
$Y_1E'_2$	$O_1T_1E'_2I'K'$ ##		
$Y_1E'_1$	$O_1T_1E'_3K'$		
E'_1 ***	T_1		
E'_3 **	$T_1Y_1E'_3$		
$D'E'_3$	I'*#*		
$O_3D'E'_3$	PI'*		
O_3#*	PQI'		
$O_3J'K'$ ***	BI'*#		
O_3J'	O_1I' ##		
$O_1Y_2J'K'$	Y_1D'*		
$O_1Y_1E'_3$	$Y_1D'E'_3$		
O_1Y_2A' *	O_3#*		
$O_1A'E'_3$	b#*		
O_1A'			
O_1#*			
$O_1T_1E'_3$*#*			
O_2			
PY_1			
PI'*			
I'*#*			
BI'*#			
E'_1I'			
E'_3I'			
b#*			

* Groups found in both Holstein-Friesians and Guernseys; ** found in Holsteins and Jerseys; *** found in Holsteins and Brown Swiss; # found in Jerseys and Guernseys; ## found in Guernseys and Brown Swiss; *# found in Holsteins, Jerseys and Guernseys; #* found in Holsteins, Guernseys and Brown Swiss; *#* found in all four of the breeds studied.

TABLE 12

The segregation of C alleles in 35 bulls with the numbers of offspring in each of the contrasted classes.

	Holstein-Friesian
H1	$C^{C_1}(19)/C^{X_2}(23)$
H2	$C^{W}(13)/C^{X_2}(10)$
H4	$C^{X_1}(32)/C^{X_2}(33)$
H5	$C^{C_1}(24)/C^{W}(24)$
H7	$C^{X_2}(\ 5)/C^{c}(10)$
H8	$C^{C_1X_1}(11)/C^{X_2L'}(7)$
H10	$C^{C_1}(27)/C^{X_2}(20)$
H11	$C^{C_1}(25)/C^{W}(20)$
H14	$C^{RWX_2}(13)/C^{X_2L'}(9)$
H15	$C^{C_1W}(\ 5)/C^{c}(13)$
H16	$C^{X_2}(\ 6)/C^{c}(7)$
H17	$C^{C_1}(\ 5)/C^{W}(8)$
H18	$C^{W}(11)/C^{X_2}(13)$
H19	$C^{C_1}(21)/C^{C_1X_1L'}(13)$

	Guernseys
G1	$C^{C_1X_1}(\ 3)/C^{c}(7)$
G2	$C^{C_1W}(\ 9)/C^{X_2}(4)$
G3	$C^{C_1R}(12)/C^{WX_2}(7)$
G4	$C^{C_1WX_2}(14)/C^{X_2}(13)$
G5	$C^{W}(13)/C^{c}(15)$
G6	$C^{W}(\ 9)/C^{X_2}(5)$
G7	$C^{C_1RW}(\ 7)/C^{W}(6)$
G8	$C^{W}(\ 9)/C^{X_2}(5)$
G11	$C^{C_2WX_1}(11)/C^{c}(9)$
G12	$C^{C_1WX_2}(\ 2)/C^{c}(2)$
G13	$C^{X_2}(\ 1)/C^{c}(1)$
G14	$C^{C_2W}(\ 9)/C^{WX_1}(7)$
G15	$C^{C_1WX_2}(\ 2)/C^{W}(5)$
G17	$C^{C_1WX_2}(\ 5)/C^{W}(3)$
G18	$C^{C_1X_1}(\ 5)/C^{c}(5)$
G20	$C^{X_2}(\ 1)/C^{c}(2)$

	Brown Swiss
S1	$C^{C_1W}(\ 3)/C^{C}(5)$

	Jerseys
J1	$C^{C_2RX_1}(\ 7)/C^{W}(3)$
J2	$C^{C_1WX_2}(\ 3)/C^{W}(7)$
J3	$C^{C_2RX_1}(\ 6)/C^{W}(6)$
J4	$C^{C_1WX_2}(\ 9)/C^{C_1W}(6)$

group $B_{O_3J'K'}$, the O_3 reaction of bloods with the groups B_{O_3} and $B_{O_3D'E'_3}$, the Q reaction of the group $B_{1QE'_1}$ and the D′ reaction of the group $B_{BO_1Y_2D'}$, are occasionally missed in the initial serological tests. Check-tests have invariably upheld the genetic criterion for positive reactions. Unfortunately, it was not possible to carry out check-tests in all these cases. It is well to mention that several of the rarely encountered groups listed in table 11 may have been serological artifacts consequent on erroneous reactions. The development of more and better reagents for the differentiation of certain of these groups of the B system, in particular the numerous genetic subtypes, constitutes a major endeavor in itself.

THE C SYSTEM OF BOVINE BLOOD GROUPS

A second system of bovine blood groups called the C system was recognized simultaneously with the B system. A detailed account of the C system, however, is not given in this report since the technics of analysis and the genetic and serological aspects of the behavior of this system are similar to

TABLE 13

The genetic groups of C factors recognized in four breeds of dairy cattle.

Holstein-Friesians	Guernseys	Brown Swiss	Jerseys
C_1	C_1	C_1	C_1W
C_1R	C_1R	C_1R	C_1WX_2
C_1RW	C_1RW	C_1RW	C_2RX_1
C_1W	C_1W	C_1X_1	W
C_1X_1	C_1WX_2	C_1X_2	c
C_1X_1L'	C_1X_1	W	
C_1X_2	C_1X_2	X_2	
C_2	C_2R	c	
C_2R	C_2W		
C_2W	W		
RWX_2	WX_2		
W	WX_1		
WX_2	X_1		
X_1	X_2		
X_2	L'		
X_2L'	c		
L'			
c			

those discussed in detail for the B system. The seven factors C_1, C_2, R, W, X_1, X_2 and L′ are members of the C system. As in the B system, these seven factors of the C system appear as the serological constituents of a number of distinct, heritable groups. In table 12 are summarized the *C* alleles which have segregated in those sires that proved to be heterozygous, showing within parentheses the numbers of offspring with each allele. The ratios obtained are in agreement with those expected of segregating pairs of alleles. Nineteen C groups have been recognized in this phase of the study. (The group $C_{C_2X_1}$, detected in the study of the C factors inherited only from S5 in his progeny, is not represented by its allele in table 16, since S5 was homozygous.) Only three additional groups were detected in the study of the C factors inherited from the maternal parents alone. The distribution of these 22 C groups by breeds is given in table 13. Although estimates of the frequencies of the *C* alleles are not available at present, differences in the breeds were noted.

From the serological point of view, less difficulty with so-called false-negative reactions has been encountered in typing the C groups than in the tests for the B groups. As in the B system, however, the problem of developing reagents for the differentiation of the many genetic subtypes is a formidable one.

DISCUSSION

Early in the development of the branch of biology called immunogenetics, IRWIN and COLE (1936) and HALDANE (1938) postulated that the antigens of erythrocytes represent virtually the immediate products of the genes that control them. The main basis for this postulate was the rarity of interaction,

if any, of genes at different loci, in controlling the specificity of cellular antigens. The epigrammatic "one gene, one enzymatic specificity," currently debated by biochemical geneticists, has had its assumed parallel in the field of immunogenetics: "one gene, one antigen."

There are two bases upon which this postulated one-to-one relationship between gene and antigen might be scrutinized. First, are genes at more than one locus involved in the control of any particular antigenic specificity? The answer to this question, on the basis of extensive studies of a variety of species, seems at present to be reasonably secure. With rare exceptions, a unit difference, as defined by the serologist, appears to depend upon a gene or genes at a single locus. If the alternatives C or no-C, G or no-G, I or no-I, etc., are examined as separate pairs of alternatives, each alternative has always been found to behave as a monofactorial difference in inheritance. The evidence upon which was based the original postulate of a direct relationship between gene and antigenic specificity has therefore been strengthened through the intervening years.

The other aspect of the one-to-one relationship has become more debatable. Is more than one antigenic specificity imposed by any particular gene? Here, the serological complexity of most of the B and C groups of cattle, for example, would seem to indicate a clear answer in the affirmative. The *B* alleles, on the basis of what might be considered a standard serological definition of antigens or antigenic components, appear to control at least 21 different antigenic characteristics, and those at the C locus at least seven. On this basis, in cattle and in several other species now believed to have similar systems of blood groups, we might be inclined to generalize: "one gene, several distinguishable serological characteristics, but each unit-specificity usually associated with only a single locus."

At least four different explanations of the apparent deviation from a one-to-one relationship between gene and antigenic specificity might be suggested. These are not all mutually exclusive, and the truth might lie in a combination of two or more of them.

(1) The genetically implied loci in question may not be units, but each might be subdivisible into a series of contiguous loci having genes with immunochemically separable effects on a single characteristic, the antigenic structure of the red blood cells. This kind of explanation is involved in the "C-D-E" symbolism of the Rh blood groups of man. In the absence of any direct genetic evidence for the divisibility of such an implied gene-complex, this has the effect of postulating a single specificity as a definitive characteristic of an antigen and its controlling genetic unit. It therefore asserts the correctness of the one-to-one hypothesis, and evades the deviation from this relationship by further postulating that the multiple specificities are controlled by a coordinate mosaic of closely linked genes.

(2) A gene may be subject to precise modification through mutation at any of a number of different points. One limited area on a *B* allele, for example, may be concerned with the specificity detected serologically in the

ultimate gene-product as G, another area with the specificity called I, and so on. Such an explanation would retain the genetic unity of the locus involved, but would maintain the separateness of the blood factors as distinct products in themselves. This explanation represents a departure from the one-to-one relationship in that it implies more than one antigen as the product of most of the controlling genes.

(3) The determination of antigenic specificity might be removed more than a single step from immediate gene action, and the effects of a single allele may involve interactions in the intervening chain of reactions. For example, an allele at the *B* locus may first control the specificity of an enzyme capable of interaction with a certain fraction of an available spectrum of distinguishable substrates, while the enzyme controlled by another allele at this locus may react with a somewhat different fraction of this spectrum. If the specificity of an eventual product depended in some degree upon the precise substrate involved in a particular reaction, a variety of ultimate antigens might result from a single primary gene product, and this variety might differ from one allele to another. Similar models could be conceived. In general, they have the disadvantage of anticipating some order of interaction among genes at different loci, in the control of any particular antigen. Subsidiary hypotheses might be constructed to negate this objection.

(4) The variety of separate specificities in any one system, as B or C, might represent only the overlapping serological properties of a series of closely related antigens controlled by the alleles of a single gene. This explanation turns to the immunological side of the hybrid field of immunogenetics for the resolution of the serological complexity. These phenomena can be readily understood on the basis of serological cross reactions alone. It is not necessary to construct models to illustrate this point; well authenticated examples, involving not only artificially conjugated antigens but natural antigens as well, are to be found in the literature (see LANDSTEINER 1945 and KABAT and MAYER 1948 for examples as well as further references to the literature).

Although the inheritance data alone do not rule out any one or any combination of the above orders of explanation, the present authors are inclined to emphasize the fourth point in this discussion, and to consider the multiplicity of antibodies evoked by a single antigen as critically involved in the interpretation of the relationship of the blood factors and the blood group systems as here defined. The authors differ somewhat in the degree of positivity with which they hold to this emphasis on a single sort of explanation.

The present results are unique only in respect to the complex serological nature of most of the blood groups of the B and C systems, and the large number of recognized groups. Parallels are to be found in other species in which antibodies produced by iso-immunization have been used in blood typing. Compare, for example, the Rh or so-called CDE groups of man (see references in WIENER, SONN-GORDON and HANDMAN 1947 and RACE, SANGER and LAWLER 1948), the A groups of Muscovy ducks and of their

hybrids with Mallards (McGIBBON 1945) and the A groups of chickens (BRILES, IRWIN and McGIBBON 1950). In spite of the ambiguities occasionally encountered in serological tests and the problem of ruling out illegitimate offspring, there has been no authenticated example of recombination of the factors in any of the blood group systems of these species. As STRANDSKOV (1948) has pointed out in respect to the Rh groups of man, there would have to be a number of well authenticated examples of factor-recombinations before these could be taken as evidence for linkage.

SUMMARY

Evidence is presented for two genetically independent systems of bovine blood groups called B and C. Of 38 blood factors that were studied, 21 are members of the B system and seven are members of the C system. All except one of the remaining 9 blood factors are excluded from the B and C systems. Eighty blood groups are recognized in the B system and 22 in the C system.

It is proposed that the blood factors of the two systems can be regarded at present as symbols for the patterns of cross reactions among single substances, the blood groups of a system. For example, the blood factors B, G, I, etc., appear as the serological integrants of B groups $B_{BGIO_1T_{2A'}}$, $B_{BGKE'_2}$ B_{BI}, B_{GI}, B_G, etc., rather than as discrete genetic characters in themselves. According to these observations, the blood groups rather than the blood factors are the inherited characteristics, and the physical reality of the blood factors as specific, particulate and independent "unit antigens," as they were previously regarded, is questionable.

The groups of the respective systems appear to be controlled by multiple alleles. A minimum of 80 alleles is indicated in the *B* series and 22 in the *C* series.

ACKNOWLEDGMENT

MISS M. CLAIRE BUSK, MR. RICHARD PIERCE, and MRS. M. W. YCAS have rendered considerable technical assistance to this study. The generous cooperation of the late HOWARD CLAPP, herdsman, PABST FARMS, Oconomowoc, Wisconsin, of GUY S. HARMON, manager, BLAKEFORD FARM, Queenstown, Maryland, and that of numerous workers at experiment stations in various states, of breeders of cattle and of the officers of the HOLSTEIN-FRIESIAN ASSOCIATION and the AMERICAN GUERNSEY CATTLE CLUB, has been a necessary factor in obtaining the blood samples. Particular appreciation should be extended to DR. G. W. BARRETT of the DAIRY HUSBANDRY DEPARTMENT of the UNIVERSITY OF WISCONSIN, for his expenditure of considerable time and effort in the arrangements for and the collection of blood samples.

LITERATURE CITED

BRILES, W. E., M. R. IRWIN and W. M. McGIBBON, 1950 On multiple alleles effecting cellular antigens in the chicken. Genetics **35**: 633–652.

FERGUSON, L. C., 1941 Heritable antigens in the erythrocytes of cattle. J. Immunol. **40:** 213–242.

FERGUSON, L. C., C. STORMONT and M. R. IRWIN, 1942 On additional antigens in the erythrocytes of cattle. J. Immunol. **44:** 147–164.

HALDANE, J. B. S., 1938 Essay in "Perspectives in Biochemistry," edited by Joseph Needham and D. E. Green. 361 pp. Cambridge: at the University Press.

IRWIN, M. R., and L. J. COLE, 1936 Immunogenetic studies of species and of species hybrids in doves, and the separation of species-specific substances in the backcross. J. exp. Zool. **73:** 85–108.

KABAT, E. A., and M. M. MAYER, 1948 Experimental Immunochemistry. xv + 567 pp. Springfield, Illinois: Charles C Thomas.

LANDSTEINER, K., 1945 The specificity of serological reactions. xiv + 310 pp. Cambridge, Massachusetts: Harvard University Press.

MCGIBBON, W. H., 1945 Further division of contrasting antigens in species hybrids in ducks. Genetics **30:** 252-266.

OWEN, R. D., C. STORMONT and M. R. IRWIN, 1947 An immunogenetic analysis of racial differences in dairy cattle. Genetics **32:** 64–73.

RACE, R. R., R. SANGER and S. D. LAWLER, 1948 Allelomorphs of the *Rh* gene *C*. Heredity **2:** 237–250.

STORMONT, C., 1950 Additional gene-controlled antigenic factors in the bovine erythrocyte. Genetics **35:** 76–94.

STORMONT, C., M. R. IRWIN and R. D. OWEN, 1945 A probable allelic series of genes effecting cellular antigens in cattle. (Abstract) Genetics **30:** 25–26.

STRANDSKOV, H. H., 1948 Blood Group Nomenclature. J. Hered. **39:** 108–112.

TOPLEY, W. W. C., 1933 An Outline of Immunity. vii + 415 pp. Baltimore, Md.: William Wood & Company.

WIENER, A. S., 1943 Blood Groups and Transfusion. 3d ed. xix + 438 pp. Springfield, Illinois: Charles C Thomas.

WIENER, A. S., E. B. SONN-GORDON and L. HANDMAN, 1947 Heredity of the Rh blood types. VI. Additional family studies, with special reference to the theory of multiple allelic genes. J. Immunol. **57:** 203–214.

KINETICS OF GENETIC RECOMBINATION IN ESCHERICHIA COLI[1]

THOMAS CLIFFORD NELSON[2]

Department of Zoology, Columbia University, New York

THE recent work of TATUM and LEDERBERG (1947) and LEDERBERG (1947) has provided evidence for a sexual mode of reproduction in the bacterium *Escherichia coli* strain K 12. When two unlike mutant substrains, each carrying a pair or more of genetic markers causing nutritional deficiencies, are mixed in minimal agar colonies appear which are prototrophic, or, like the wild type, capable of growth in minimal medium. This has been interpreted as being due to a fusion of the parental bacteria, that is, syngamy, with subsequent segregation and recombination of the genetic factors. Recombination classes other than the prototrophs were expected and found by supplementing the minimal agar with appropriate growth factors. Recombination between these and other factors has allowed a linkage map to be constructed.

If syngamy does occur a diploid phase would be expected. Owing to the low frequency of the prototroph-forming process, the actual fusion of parental cells, if it occurs, would be difficult to observe. No attempts in this direction have been reported. However, a persistent heterozygote has been found by LEDERBERG (1949) which offers contributory evidence for considering the recombination classes to be the result of syngamic fusion and segregation.

In view of the importance of the hypothesis of bacterial syngamy, alternative hypotheses must be considered carefully. In addition further lines of evidence may arise from studies of the consequences to be expected from a syngamic mode of reproduction.

Three alternative hypotheses must be considered; syntrophy, back-mutation, and transformation. The hypothesis of syntrophy would consider the prototroph colonies as mixtures of the two parental types, each type supplying the growth factors it is able to synthesize to the other type which is unable to synthesize them. By this hypothesis only the parental type cells would be expected in the prototroph colony. Among other results the finding of the other recombination classes rules out this interpretation. The hypothesis of back-mutation considers the prototroph colonies to arise by multiple mutation. Control platings of the parental cells separately show no multiple back-mutants, an event which may be expected to occur with too low a frequency (as determined by compounding single back-mutation rates) to explain the

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University.

[2] Present address: Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena 4, California.

GENETICS 36: 162 March 1951.

number of prototroph colonies recovered. A combination of these two hypotheses, suggested by RYAN and SCHNEIDER (1949), has not been ruled out as an explanation for at least some of the prototrophs. This hypothesis considers a limited growth of the parental type cells to occur aided by syntrophy or slow growth. Thus microcolonies would form in which, due to the large number of cell divisions involved per plate, multiple back-mutation could be expected to occur. This hypothesis will not explain all of the phenomena which have been observed (the persistent heterozygote) but possibly errors in linkage values may be due to the occurrence in microcolonies of multiple back-mutation in addition to syngamy. The final alternative hypothesis considers the prototrophs to be formed by the action of multiple transforming principles similar to those discovered in pneumococcus by AVERY, MACLEOD and MCCARTY (1944) and in *Escherichia coli* by BOIVIN (1947). No such action or factors have been found by LEDERBERG (1947) whose experiments lead to a second line of evidence supporting the hypothesis of syngamy.

Advantage may be taken of the low frequency of the prototroph-forming process, as demonstrated by LEDERBERG (1947), HAAS, WYSS and STONE (1949), and CAVALLI and HESLOT (1949), to determine whether or not it takes place in the quantitative manner to be expected of gametic fusion. In the conjugation process of syngamy the frequency of zygotes should be proportional to the product of the concentrations of the gametes, that is, to the frequency with which conjugable gametes find themselves either in contact or within a limited distance of each other, and to the time during which interaction is possible. A mathematical expression relating the concentration of parental cells and the yield of prototrophs will be described below. Data will be presented which test the resultant expression and thus the hypothesis of bacterial syngamy.

A similar investigation has been initiated by KANN (1949). KANN's attempts to substantiate the relationship by plate methods were not successful due to syntrophy between the mutants employed. That a threshold relationship does hold between prototrophs and parental bacterial concentrations was shown by making crosses in minimal liquid medium.

THEORY

If gametic fusion occurs between two types of cells it would be expected to follow a biparticulate or " bimolecular " frequency distribution. The relationship between the number of parental cells mixed and the number of recombinants obtained (as a measure of the number of fusions occurring) may be derived in two ways; (1) by a consideration of the spatial distribution of parental cells immobilized in agar if fusion occurs in agar, or (2) by a kinetic analysis of colliding parental cells if fusion occurs in liquid suspension. Both expressions reduce to a linear function of the product when the time variable is eliminated from the kinetic equation. Evidence will be presented to indicate that the primary event in initiating recombination in

these experiments occurs during the mixing of liquid suspensions of parental bacteria and not in the agar.

The kinetic analysis considers the formation of zygotes to occur through the collision of parental bacteria. Hence the number of zygotes obtained should be a function of the time during which free movement of the parental bacteria is possible. The rate of formation of zygotes, dZ/dT, would then be proportional to the numbers N_1 and N_2 of the two different parental bacteria. This is similar to a bimolecular reaction in chemical kinetics:

$$dZ/dT = K(N_1 - Z)\ (N_2 - Z) \qquad (1)$$

Since Z in these experiments is negligible compared to N_1 or N_2 a zero order equation is obtained:

$$Z = K\, N_1 N_2\, T \qquad (2)$$

The number of zygotes is not directly determinable but must be measured in terms of some recombination class. Hence not every zygotic fusion will be detected. Certain recombination classes are easier to screen. Prototrophs have been used here. Drug and chemical resistance have also been used by LEDERBERG (1950). The number of prototrophs, p, will be related to the number of zygotes, Z, by the approximate exchange frequency, r, of the factors involved in the cross. Thus $rZ = p$. The reaction rate constant is a direct measure of the syngamic potential and should be used in preference to empirical frequencies (HAAS, WYSS and STONE 1949 and CLARK, STONE, WYSS and HAAS 1950). Thus the recombination phenomenon can be investigated and expressed in a quantitative manner.

The quantitative expressions expected on the basis of alternative hypotheses discussed previously depend upon assumptions specific for each case. Expressions set up under different sets of assumptions can give forms similar to the kinetics equation. Thus the experimental confirmation of the kinetics equation derived above does not offer critical proof of syngamy. Such a finding would however be consistent with bacterial syngamy and would lay the groundwork for the quantitative investigation of controlled environmental and cultural conditions on the phenomenon.

METHODS

Mutant substrains of *Escherichia coli* strain K 12 were used. The sources and requirements of these mutants are listed in table 1. These stocks were maintained by monthly transfer onto 2 percent nutrient agar slants and stored at 5°C.

Standard bacteriological sterile technique was used throughout. Difco agar was washed according to the procedure of RYAN and SCHNEIDER (1949) followed by an additional washing in boiling alcohol for ten minutes. All glassware was washed in hot soap solution followed by chrome sulfuric acid.

Cultures were started from slants by inoculation into 2 percent nutrient broth contained in 12-ml centrifuge tubes. All cultures were rolled at 38 ± 1 degrees centigrade by the procedure developed by RYAN (private communica-

TABLE 1

Mutant substrains of Escherichia coli K 12

Mutant	Source	Requirements	Remarks
679–680	E. L. Tatum (1945)	Leucine and threonine	
Y 24	J. Lederberg (1947)	Cystine and phenylalanine	The biotin requirement was not detected.
Y 161	D. Bonner	Histidine and methionine	
175–12	E. E. Kann (1949)	Tryptophane and adenine	
p^-	K 12	Proline	These substrains were detected after ultraviolet irradiation in this laboratory by Mr. Malcolm H. Schvey.
$px^=$	p^-	Proline and unknown factor	
m^-	K 12	Methionine	
$mt^=$	m^-	Methionine and tryptophane	This substrain was detected after ultraviolet irradiation in this laboratory by Mr. Samson Gross.

tion). Cultures varying in age from 9 to 16 hours have been used. Younger or older cultures give fewer prototrophs. The cultures were thoroughly washed 3 to 5 times by centrifugation and resuspension in 0.9 percent sterile saline before use.

For detection of prototrophs samples of the washed inocula were plated in synthetic medium according to RYAN and SCHNEIDER (1949) containing 0.5 percent glucose and 1.5 perçent washed agar (2 X concentrated salt medium and distilled water containing the requisite amounts of glucose and agar were autoclaved separately and mixed just before use to prevent flocculation). Distilled water and Merck reagent grade chemicals were used throughout. Media were not remelted and used after once setting. Special care was taken to prevent contamination of minimal media by chemical supplements. A 10-ml bottom layer of minimal medium containing agar but no glucose was delivered by pipette into standard 10-cm Petri plates. The volumes of the inocula and suspending saline were also carefully measured except in the kinetics experiments, the exact volume depending upon the design of the individual experiment. The volume V and its parameters (surface area and depth), that is, the plate geometry, were thus kept constant. The plates were incubated at $38 \pm 1°C$ for varying lengths of time depending upon the individual experiment. The prototroph colonies were then counted under a binocular microscope using an electric recording pen. Colonies were periodically isolated and tested for the prototroph characteristic by streaking on minimal agar and for the coliform reaction on Endo agar. Supplemented agar was prepared by the addition of the proper amino acids at a concentration of 50 gammas/ml to the glucose and agar before autoclaving.

Total numbers of cells were determined by appropriate dilution in sterile 0.9 percent and plating in 2 percent nutrient agar containing 0.5 percent glucose.

RESULTS

Relationship between prototroph numbers and parental bacterial concentrations

To test whether the relationship $p \alpha N_1N_2$ holds where the time and volume of mixing are held constant experiments similar to that described by figure 1 were performed. In this experiment different volumes of washed inocula of substrains 679–680 and Y 24 were placed on bottom-layered Petri plates in separate drops. These volumes were varied simultaneously and equally; thus a N_1N_2 value of 0.1 represents 0.1 ml of the 679–680 inoculum plus 0.1 ml of the Y 24 inoculum, a N_1N_2 value of 0.4 represents 0.2 ml of each inoculum, a N_1N_2 value of 0.9 represents 0.3 ml of each inoculum, etc. The ratio of the volumes, and therefore the ratio of the numbers of parental bacteria, were kept constant by this method. Sufficient saline was added to bring the volume to 5.00 ml when mixed. In order to obtain random distribution of the bacteria the combined inocula and saline were thoroughly mixed by rotation. Thus there was ample chance for collision between the parental bacteria. In series 1 10.0 ml of 1.5 X molten agar containing glucose were added immediately by pipette and thoroughly mixed with the combined inocula and saline. In the other series varying lengths of time were allowed before a like volume of agar was added and mixed. The mixed inocula and saline were periodically swirled. The results show a linear relationship between prototroph numbers and the product of the parental bacterial concentrations and an increased production of prototrophs with time of mixing.

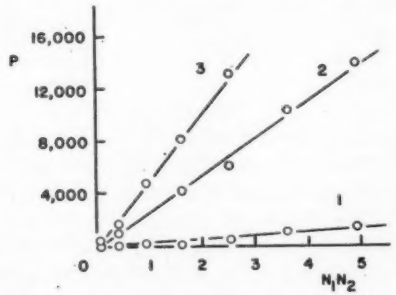


FIGURE 1.—Linear law and time of plating. P is the number of prototroph colonies per plate and N_1N_2 is directly proportional to the product of the parental bacterial concentrations; 2.5 equalling 0.96×10^9 679–680 times 0.99×10^9 Y 24 cells per plate. Series 1 was plated immediately after mixing and has a slope value and standard error of $7.7 \pm 0.7 \times 10^{-16}$, series 2 at 90 minutes with a slope of $82 \pm 4 \times 10^{-16}$, and series 3 at 120 minutes with a slope of $138 \pm 1 \times 10^{-16}$. The slope values were calculated by linear regression of P on N_1N_2 and the standard error of the linear regression coefficient is reported as the error.

Expression (2) may be tested in other ways. The ratio of parental bacterial concentrations may be expressed as t where $t = N_1/N_2$. In a cross in-

volving different values of t the parameter p/N_2^2 should be a linear function of t. The results shown in figure 2 demonstrate the essential validity of this relationship with, however, a departure from linearity at high values of the ratio. This may be due to syntrophy between the parental bacteria which occurs only at high ratios (KANN 1949) thus giving rise to more than the expected yield of prototrophs or may be a complication arising from intratype mating. In addition, for an equal number and ratio of parental bacteria the number of prototrophs obtained per plate should be an inverse function of the volume of the agar, that is: $p_1/p_2 = V_2/V_1$. Figure 3 demonstrates that this relationship holds. The 16-ml series has a slope not significantly different, by STUDENT's t test at the 5 percent level, from one-half the slope of the 8-ml series.

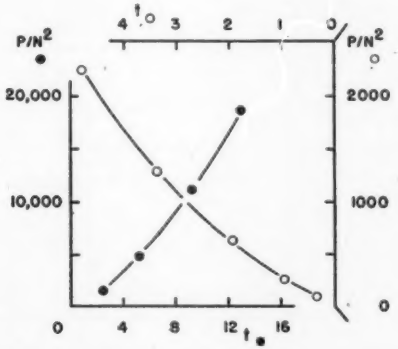


FIGURE 2.—Ratio test. The ordinate represents the parameter $P/N^2 \times 10^{10}$. The abscissas represent the t ratios. The solid circles give t equal to Y 24/679–680 where N is 679–680. The open circles give t equal to 679–680/Y 24 where N is Y 24. Each point is the arithmetic mean of five or more plates containing varying concentrations of parental bacteria (all of the same t ratio).

Similar results have been obtained for crosses between other substrains under conditions where inhibition of prototroph colonies by parental bacteria is not operative and where linkage relationships allow the formation of sufficient numbers of prototrophs. For most substrains, however, the number of prototroph colonies fails to increase at high concentrations of parental bacteria, the resultant saturation level depending upon the specific substrain. The size of the individual prototroph colonies decreases and, at the highest concentrations of parental bacteria, no prototroph colonies are visible. Any prototrophs that may have been formed or are added in reconstruction experiments merge into the general clouding of the plate due to the heavy suspension of the parental bacteria. This is demonstrated in figure 4 for a cross of px$^-$ × 679–680.

The crosses possible with the substrains listed in table 1 may be roughly seriated in decreasing order of their slope values when p is determined as a

function of N_1N_2. Thus the cross $p^- \times Y\ 24$ stands at the top of the list, $Y\ 24 \times 679\text{–}680$ and $Y\ 24 \times px^=$ are intermediate, and $Y\ 161 \times 175\text{–}12$ is at the bottom. The reason for this order may most probably be ascribed to different values of the linkage factor, r. Conditions of growth, that is, age of the culture (within the 9- to 16-hour limit) are of secondary importance providing the time of mixing is kept constant.

Chemical supplementation of minimal medium to obtain single or multiple factor non-parental recombination classes has been performed. The slope values are not constant between separate but similarly performed experiments thus invalidating this technique as a method of measuring relative linkage distances. The relative order of factors does remain constant and consistent.

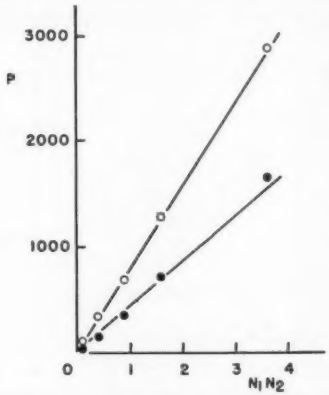


FIGURE 3.—Volume effect. P is the number of prototroph colonies per plate and N_1N_2 is directly proportional to the product of the concentrations of the parental bacteria; 1 equalling 1.4×10^8 679–680 times 0.83×10^8 Y 24 cells per plate. The open circles are experimental points for a series of plates of 4.0 ml suspending volume + 4.0 ml 2 X agar = 8.0 ml total volume. The resultant slope and standard error are 0.78 ± 0.01. The closed circles are experimental points of 8.0 ml suspending volume + 8.0 ml 2 X agar = 16.0 ml total volume. The resultant slope and standard error are 0.44 ± 0.02. The same cultures and mixing times were used for both series of plates.

Substrains Y 24 and 679–680 were chosen for further studies. All following experiments relate to the crossing of these substrains.

Characteristics of the plate detection method for prototrophs

Certain characteristics of the plate detection method for screening prototrophs were investigated to ensure the validity of the technique. The following results were obtained concerning the secondary crop of prototrophs which appear on plates incubated longer than 24 hours:

(a) Counts of the number of prototroph colonies per plate as a function of the time since plating were found to increase by 10 to 50 percent and reach a maximum at about 50 hours.

(b) The size of the prototroph colonies, as determined with an ocular micrometer, was found to follow a monomodal distribution at 24 hours which became bimodal after longer incubation times.

(c) Increase in the average colony size of the two distributions, as measured with the ocular micrometer, stops at the same time (about 50 hours in 0.5 percent glucose plates).

(d) The first distribution of prototroph colonies was marked at 20 hours and the second distribution differentially as it appeared from 30 hours on. Measurements of the relative sizes were then made. The smaller sized distribution of prototroph colonies was the late appearing distribution.

(e) Isolates of both distributions were grown in liquid minimal medium and plated in minimal agar. Both types of isolates gave equal sized large colonies on replating.

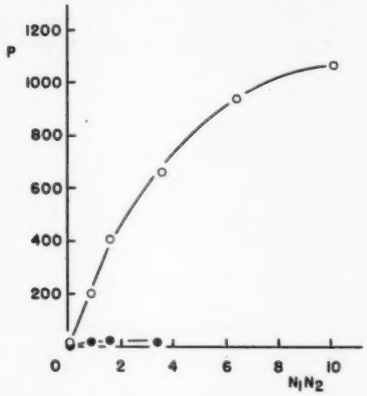


FIGURE 4.—Linear law. P is the number of prototroph colonies per plate and N_1N_2 is directly proportional to the product of the parental bacterial concentrations; 10 equalling 1.6×10^9 679–680 times 4.3×10^9 $px^=$ cells per plate for the curve represented by the sold circles and 1.4×10^9 Y 24 times 4.3×10^9 $px^=$ cells per plate for the curve represented by the open circles.

(f) A secondary distribution never appears on plates unless the primary distribution is present.

The secondary prototroph distribution probably represents a second phase of prototroph formation occurring on the plates after setting of the agar. A limited growth of the original parental bacteria might occur when the original prototrophs, represented by the earlier appearing distribution, grew to such a colony size that their released metabolites served as nutrilites for the parental bacteria. This limited growth of parental bacteria would allow further prototrophs to be formed in contiguous microcolonies as suggested by RYAN and SCHNEIDER (1949). The resultant secondary distribution of prototroph colonies would not be expected to grow to full size because of exhaustion of glucose by the primary prototroph distribution or other inhibitory

factors. After the occurrence of this secondary plate phenomenon was appreciated all counts except those reported in figure 1 were made between 20 and 30 hours.

As the number of prototroph colonies per plate and the number of parental bacteria per plate reach large values the prototroph colonies decrease in size. To investigate whether prototroph colonies are inhibited by the overwhelming concentration of parental bacteria reconstruction experiments were performed. A known number of prototrophs previously isolated from crosses formed with the same substrains were plated with the inocula of a cross. As shown in figure 5 for a cross of Y 24 × 679–680 the added prototrophs were recovered in expected yield. No time lag between the development of the added prototrophs and the newly formed prototrophs resulting from the cross was observed. Complete recovery of prototrophs plated along with high

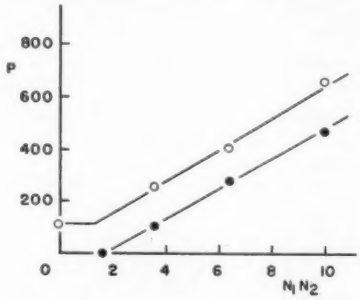


FIGURE 5.—Recovery of added prototrophs. P is the total number of prototroph colonies per plate and N_1N_2 is directly proportional to the product of the parental bacterial concentrations; 10 equalling 2.6×10^8 679–680 times 1.8×10^8 Y 24 cells per plate. No prototrophs were added to the series of plates represented by the solid circles. About 120 prototrophs were added to each plate of the series represented by the open circles. The failure of the curve to intercept the origin of the axes may be due to an induction period or an inability of all the bacteria to undergo syngamy.

concentrations of a single parental type was also obtained. At high numbers of prototroph colonies the colony size is reduced until eventually only microcolonies and clouding of the plate occur. No distinction of added from newly formed prototrophs can be made at any level of prototroph colony number. This demonstrates that, in the cross of Y 24 × 679–680, the inhibition of prototroph colony size is due to the prototroph colonies themselves and not to the parental bacteria. In other substrains, for instance the substrains illustrated in figure 4, the parental bacteria in high concentrations do inhibit the growth of prototroph colonies.

Kinetics of prototroph formation in liquid

That the number of prototrophs formed per unit volume increases with time of mixing is indicated in figure 1. In order to obtain meaningful results

on the formation of what may be termed syngamonts the rate of collision between parental bacteria had to be kept constant. This was accomplished by rolling the parental bacteria suspended in sterile minimal medium without glucose or sterile saline in flasks attached to a turntable rotating at 200 revolutions per minute. Aliquots of the suspension were then plated in minimal agar after various time intervals.

Before drawing the conclusion that any additional prototrophs found with increased time of mixing indicates that syngamy occurs in liquid suspension an alternative possibility must be eliminated. This alternative explanation postulates that all syngamy occurs in the solidified agar, possibly as a result of microcolony formation, and that mixing in liquid previous to plating merely stimulates this process. To test this hypothesis a cross of Y 24 × 679–680 was run in liquid and various dilutions plated after 40 minutes. If syngamy occurred in plates the number of prototrophs obtained per plate should be proportional to the product of the parental bacterial numbers in each dilution. If syngamy does occur in liquid then the number of prototrophs obtained per plate should be directly proportional to the volume of suspension plated. That this latter event occurs is shown by figure 6. This experiment also

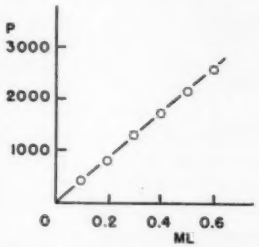


FIGURE 6.—Test of occurrence of syngamy in liquid. P is the number of prototrophs in samples of the volumes given on the abscissa in milliliters (ML) from a cross of 3.2×10^8 Y 24 times 4.1×10^8 679–680 cells in 5.0 ml of saline rolled for 40 minutes at 200 cycles per minute.

demonstrates that plate syntrophy or slow growth is a negligible factor, if present, in prototroph formation in liquid (not counting the secondary prototrophs appearing later on the plates). Platings of the parental bacteria in nutrient agar at the beginning and end of mixing were equal within plate sampling error. This eliminates the possibility of appreciable growth of the majority of the parental bacteria in liquid.

With this point established experiments could be undertaken to test the dependence of prototroph frequency on time of mixing. The results presented in figure 7 substantiate the proportionality of prototroph frequency with time. This type of experiment has been repeated a number of times and two additional phenomena have been encountered. After long periods of mixing, ranging up to 6 hours, the concentration of prototrophs fails to increase in some experiments—saturation occurs. In suspensions containing less than 10^8 bac-

teria per 20 ml an induction period occurs. These phenomena are illustrated for two separate experiments by figure 8.

Control experiments

Various controls have been run to test the hypotheses alternative to bacterial syngamy. Platings of double mutants separately in minimal medium have never given rise to prototrophs. However platings of single mutants in minimal medium or double mutants in medium supplemented with one required growth factor have given single reversion types. The hypothesis of syntrophy or slow growth with reversion is more difficult to test. Ideally, mixtures of the parental bacteria in agar should be recovered by dissolution of the agar. Since this is technically impossible without killing the bacteria mixtures of the parental bacteria were spread on the surface of minimal agar plates. Numbers (10^5 to 10^7 of each type) of the parental bacteria were used

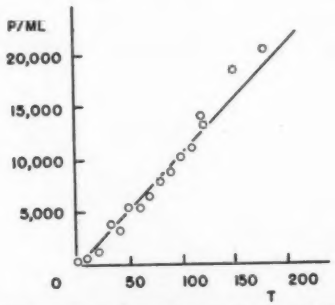


FIGURE 7.—Kinetics of prototroph formation in liquid. P/ML is the number of prototrophs per milliliter as a function of T, the time of mixing in minutes. The cross was run in saline in sterile 125-ml Erlenmeyer flasks rotated at 200 cycles per minute. A bottom layer of 1.5 percent washed agar solidified in 10 ml of minimal medium lacking glucose was present. 2.0×10^9 Y 24 and 2.0×10^9 679–680 cells were used in a total liquid volume of 20.0 ml.

which gave few or no prototrophs as detected by differential plating in minimal and nutrient agar. After varying time intervals the bacteria were scraped off, diluted, and plated. Within the rather wide limits of error due to the cumulative series of manipulative procedures no significant syntrophy or slow growth was detected. Instead, death of the cells occurred. Because of the lower numbers of parental cells used in these experiments the method cannot be interpreted as a completely fair test of syntrophy.

No prototrophs have been obtained in attempted crosses in which the two parental types were plated in separate agar layers separated by a thin intermediate layer. Lastly, platings of a marked mutant of a strain other than K 12, a lactose non-fermenting mutant of the histidineless substrain of *Escherichia coli* 15 used by RYAN and SCHNEIDER (1949) prepared by DR. MARGARET LIEB, with mutants of K 12 have given no significant increase in

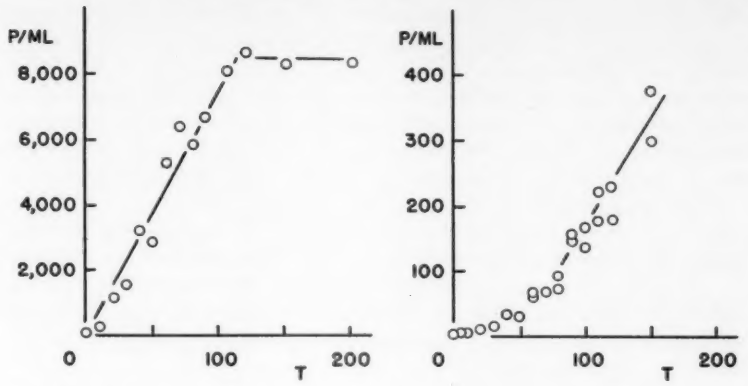


FIGURE 8.—Saturation and induction period in crosses in liquid. Both figures represent the number of prototrophs formed per milliliter (P/ML) as a function of the time of mixing in minutes (T). Two different experiments are represented. The total volume in each experiment was 20.0 ml and the rate of mixing was 200 cycles per minute. Saturation is shown by the curve on the left in which the parental bacterial concentrations were 2×10^8 679–680 cells and 2×10^8 Y 24 cells per 20.0 ml. An induction period is demonstrated by the curve on the right in which the bacterial concentrations were 0.40×10^8 679–680 cells and 0.40×10^8 Y 24 cells per 20.0 ml. A bottom layer of 1.5 percent washed agar solidified in minimal medium lacking glucose was present.

the number of prototrophs after corrections for reversion of the single mutant were made. All such prototrophs carried the lactose negative marker.

DISCUSSION

The results presented in this paper add further weight to the concept of bacterial syngamy by demonstrating the proportionality of the number of prototrophs to the concentration of parental bacteria and, under certain conditions, to time of mixing. Since the prototroph formation constant (the slope obtained by plotting the number of prototrophs against the product of the parental bacterial concentrations) is variable between different experiments, even with constant mixing conditions, it is probable that phases in the population growth cycle exist in which the bacteria show different capabilities of undergoing syngamy. By the use of chemically defined media in which the growth phases are experimentally determinable it should be possible to obtain bacteria in a growth phase in which the syngamic potential, as measured by the rate and extent of formation of prototrophs, is a maximum and consistent between experiments. The effect of various experimental conditions upon syngamy may then be determined and quantitatively expressed.

The methods and results described in this paper may not measure syngamy proper but a primary event, possibly auto-agglutination of the bacteria, required to bring sufficient numbers of bacteria together in intimate contact.

The process of syngamy is not necessarily completed in liquid medium in the kinetics experiments. Syngamy might occur in the agar subsequent to the mixing and agglutination of the bacteria in the liquid suspension. This interpretation was suggested by DR. J. LEDERBERG. This mechanism would explain induction as the lag time during which cell clumps are formed of a sufficiently large size such that the occurrence of syngamy becomes probable and saturation as the completion of agglutination. While no direct evidence for the existence of inhibitors or stimulators of syngamy (such as the fertilization gamones) have been obtained, the induction period is suggestive of the preparation of the medium or the bacteria by a secretion product before syngamy can occur. In more concentrated suspensions a critical concentration of the hypothetical substance would be reached more rapidly than in dilute suspensions, thus eliminating a measurable lag. Such secretions might act by facilitating agglutination.

It would be of interest to determine the average number of collisions of parental bacteria required per effective syngamic fusion. Such a calculation can be made through the use of the VON SCHMOLUCHOWSKI and SUTHERLAND-EINSTEIN diffusion equations. Assuming that the bacteria adhere to the diffusion laws, taking typical values for the size and density of the bacteria, and employing the data from the experiment described by figure 7 calculations give a value of the order of 10^4 collisions per prototroph.

SUMMARY

The kinetics of recombination of genetic factors in *Escherichia coli* K 12 have been investigated. A model based on bimolecular chemical reactions was compared with experimental results. Genetic recombinants, measured by selective screening for prototrophs, were recovered in expected yield as a function of (a) parental bacterial numbers, ratio of parental bacteria, and volume of the system, and (b) time of mixing of the parental bacteria. These findings support the concept of bacterial syngamy and allow a quantitative investigation of the phenomenon.

ACKNOWLEDGMENTS

The author wishes to express his appreciation for the interest and guidance of DR. F. J. RYAN during the course of this and other researches.

This work was completed while the author held a University Fellowship.

LITERATURE CITED

AVERY, O. T., C. M. MACLEOD and M. MCCARTY, 1944 Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J. Exp. Med. **79**: 137–158.

BOIVIN, ANDRE, 1947 Directed mutation in colon bacilli, by an inducing principle of desoxyribosenucleic nature: its meaning for the general biochemistry of heredity. Cold Spring Harbor Symp. Quant. Biol. **12**: 7–17.

CAVALLI, L. L., and H. HESLOT, 1949 Recombination in bacteria, outcrossing *Escherichia coli* K 12. Nature **164**: 1057.

CLARK, J. BENNETT, FELIX HAAS, WILSON S. STONE and ORVILLE WYSS, 1950 The stimulation of gene recombination in *Escherichia coli*. J. Bact. **59**: 375–379.

HAAS, F., O. WYSS and W. S. STONE, 1949 The effect of irradiation on recombination in *Escherichia coli*. Proc. nat. Acad. Sci. **34**: 229–232.

KANN, ELLEN E., 1949 Studies on genetic recombination among mutants of *Escherichia coli*. Thesis, Indiana University Library.

LEDERBERG, JOSHUA, 1947 Gene recombination and linked segregations in *Escherichia coli*. Genetics **32**: 505–525.

1949 Aberrant heterozygotes in *Escherichia coli*. Proc. nat. Acad. Sci. **35**: 178–184.

1950 The selection of genetic recombinations with bacterial growth inhibitors. J. Bact. **50**: 211–215.

RYAN, F. J., and L. K. SCHNEIDER, 1949 Mutations during the growth of biochemical mutants of *Escherichia coli*. Genetics **34**: 72–91.

TATUM, E. L., 1945 X-ray induced mutant strains of *Escherichia coli*. Proc. nat. Acad. Sci. **31**: 215–219.

TATUM, E. L., and J. LEDERBERG, 1947 Gene recombination in the bacterium *Escherichia coli*. J. Bact. **53**: 673–684.

THE FREQUENCY OF X-RAY INDUCED CHROMOSOME ABERRATIONS IN TRADESCANTIA AS MODIFIED BY NEAR INFRARED RADIATION [1]

HENRY T. YOST, JR.

Johns Hopkins University, Baltimore, Md.

Received June 6, 1950

PREVIOUS investigations have shown that infrared radiation is effective in increasing the number of chromosomal aberrations resulting from X-radiation (KAUFMANN, HOLLAENDER and GAY 1946; SWANSON and HOLLAENDER 1946). When used alone, however, infrared radiation has no visible effect upon the chromosome. During recent years, these radiations have been used in combination with mutagenic agents to investigate numerous problems. Although increases in chromatid aberrations have been secured in Tradescantia, the effect of infrared radiation upon the induction of chromosome aberrations has not been tested. It was felt that an extension of the investigation to cover chromosome aberrations might lead to a solution of certain problems which had arisen in the earlier studies. On the one hand, two hypotheses have been advanced to explain the mechanism of action of infrared radiation in producing its effect. One states that infrared radiation affects only the process of recombination, favoring recombinations over restitution (KAUFMANN, HOLLAENDER and GAY 1946). The other postulates that the effect is to increase the total number of primary breaks (SWANSON and HOLLAENDER 1946). As the recombination process takes place during and after X-radiation, it seemed that investigations of the effects of posttreatments with infrared radiations should help to discriminate between these two hypotheses. Tradescantia possesses certain advantages for such an investigation, since the period of recombination following X-radiation has been shown to be very short (SAX and ENZMANN 1939; SAX 1941, 1943; LEA and CATCHESIDE 1942; MARINELLI, NEBEL, GILES and CHARLES 1942). Utilization of chromosome rather than chromatid aberrations permits an extension of the period of effective posttreatments to at least 96 hours after X-radiation. If the effect of the infrared were upon the recombination process alone, posttreatment effects would be limited to the time interval established for the process in Tradescantia, that is, during the first hour after X-radiation. This did not prove to be the case, as the data presented in this paper show.

If, on the other hand, and contrary to the evidence of SAX and others, recombination of X-ray-induced broken ends can take place over a longer period of time than has been supposed, then posttreatments with infrared

[1] This work was supported in part by a grant from the NATIONAL INSTITUTE OF HEALTH to C. P. SWANSON.

radiation, given at varying times after X-radiation, should throw some light on the process of recombination. As McCLINTOCK (1941, 1942) has demonstrated in maize, the time of breakage, as it relates to recombination, is not the important factor; rather, the time at which, and the tissue within which, certain mechanically induced breaks recombine, determine between them the type of fusion which takes place. If such an hypothesis can explain the results obtained in Tradescantia, it would be expected that the increased aberrations resulting from the infrared posttreatment would be either chromosome or chromatid aberrations, depending upon whether the infrared radiation was given at a time when the chromosomes were effectively single or double. It was to test this hypothesis that these experiments were carried out.

EXPERIMENTAL PROCEDURE

Inflorescences of *Tradescantia paludosa,* Anderson and Woodson (clonal line number 5 of Sax), were used as the experimental material for this study. X-ray treatments consisted of radiation from an industrial type of instrument, delivered at 80 kilovolts and 5 or 10 milliamperes, the distance being 16 inches from the tungsten target. The window of the tube provided filtration equivalent to 1.5 mm Al. The treatment time varied from three to six minutes. The dosages, in roentgen units, are given with the data.

Buds to be treated with infrared were placed in a pyrex tube, which was rotated slowly for three hours in a beam of light emitted by a commercial 250-watt drying lamp operated at 110 volts. The radiation was filtered with a Corning glass filter No. 2540, opaque to visible wavelengths, but transmitting a high percentage of infrared from 750 mμ to 4000 mμ. The pyrex tube was cooled by a stream of water to eliminate any possibility of a general temperature rise in the buds. The glass tube and water jacket probably removed all infrared radiations longer than 1500 mμ.

In each group, all buds, whether treated with infrared or not, were X-rayed at the same time to insure adequate controls. The material was fixed in 3:1 alcohol-acetic, four days after X-radiation, regardless of the time when the infrared radiation was applied. This time was chosen since at this time only chromosome aberrations are to be observed, whereas at times earlier than this occasional chromatid breaks are found. Only metaphase figures of the first microspore division were scored for induced aberrations. Each line in the *Chromosome Number* columns of tables 1 and 2 represents an analysis of three to six slides of acetocarmine smear preparations. As only ring and dicentric chromosomes were scored as a criterion of effect, the figures presented in the tables do not represent total breakage of the chromosomes.

RESULTS

The results of pretreatment with infrared radiation are given in table 1. All pretreatments were given immediately before X-radiation. Table 2 presents those data pertaining to the effect of posttreatment with infrared radiation on the induction of chromosomal aberrations by X-rays. The post-

TABLE 1

Frequency of X-ray induced chromosomal aberrations when infrared is applied as a pretreatment.

	Approximate dose	Chromosome number	Number of breaks	% breakage	% increase	χ^2	P
X-ray	90 r	3600	48	1.33 ± 0.27	77	5.13	0.025
X-ray + infrared		3480	82	2.36 ± 0.37			
X-ray	170 r	4200	96	2.29 ± 0.32	127	21.73	< 0.001
X-ray + infrared		3096	160	5.17 ± 0.56			

treatments were given at various intervals after the X-raying, ranging from 0–3 to 93–96 hours, as indicated in the table.

In all cases, the data are summarized as a series of experiments with the X-ray control data given for each group. As fluctuations are evident between the controls of the various groups, comparisons may legitimately be made only between the data for the control and treated series of the same group. The data agree with those of SAX (1942) in that dicentrics were found to be approximately three times as frequent as ring chromosomes. No significant differences were apparent between the increases of the ring as opposd to the dicentric types of aberration. The percentage of increase induced by the infrared treatments does not show any clear relationship with the amount of delay in treatment. Inspection of the data indicates the possibility that the percentage increases induced by infrared treatment are greater with larger doses of X-rays. This suggests that since the series undergoing the greatest delay

TABLE 2

Frequency of X-ray induced chromosome aberrations when infrared is applied as a posttreatment.

Infrared (hrs. after X-ray)		Approximate dose	Chromosome number	Number of breaks	% breakage	% increase	χ^2	P
0–3	X-ray	170 r	2790	66	2.37 ± 0.41	68	6.08	0.015
	X-ray + infrared		3336	132	3.96 ± 0.48			
21–24	X-ray	90 r	2400	32	1.33 ± 0.32	100	4.75	0.033
	X-ray + infrared		1800	48	2.67 ± 0.54			
21–24	X-ray	170 r	1800	48	2.67 ± 0.54	94	7.32	0.009
	X-ray + infrared		1782	92	5.16 ± 0.73			
45–48	X-ray	250 r	3708	162	4.37 ± 0.51	62	12.14	< 0.001
	X-ray + infrared		3702	262	7.08 ± 0.59			
69–72	X-ray	250 r	3906	158	4.04 ± 0.45	51	8.16	0.007
	X-ray + infrared		3600	220	6.11 ± 0.56			
93–96	X-ray	350 r	3096	308	9.95 ± 0.75	69	29.47	< 0.001
	X-ray + infrared		3210	540	16.82 ± 0.93			

in posttreatment also received the greatest X-ray dose, the infrared effect may have a relationship with time which is obscured by the manner in which these data were collected. Unfortunately at the present time, data are not yet available to elucidate this problem fully. It may be noted, however, that SWANSON and HOLLAENDER (1946) failed to find any such relationship in their analysis of chromatid breakage. All increases in overall percentage of breakage were shown to be significant or highly significant by chi-square calculations.

DISCUSSION

The pretreatment data presented here are in agreement with the findings of KAUFMANN, HOLLAENDER and GAY (1946) on the effect of infrared treatment upon chromosomal aberrations in Drosophila, and those of SWANSON (SWANSON and HOLLAENDER 1946; SWANSON 1947; SWANSON 1949) on chromatid aberrations in Tradescantia. The data of SWANSON and HOLLAENDER showed that 0.29 percent of chromatid exchanges were induced with 47.5 r units of X-radiation as compared with 1.72 percent induced when infrared pretreatments were given; similarly, 95 r induced 2.15 percent of exchanges, as compared to 11.88 percent with infrared pretreatments. The first case shows an increase of 594 percent in exchanges, while the second shows an increase of 553 percent. Comparison of their data with those presented in table 1 shows that there is no qualitative difference in the effects of infrared treatments, when used to induce chromosome or chromatid aberrations. There are, however, some quantitative differences. The percentage increase induced by the infrared treatment is lower for the chromosome than for the chromatid exchange aberrations. There are probably two factors which enter into this: 1) the higher sensitivity of the chromosomes to X-rays at prophase, the approximate time when chromatid breaks are induced; and 2) the fact that twelve chromatids per six chromosomes are scored, although the figures listed in both cases are percentage of aberrations per six haploid chromosomes.

The posttreatment data presented in table 2 show that the infrared treatment may be given as many as 96 hours after X-radiation and still result in increases in chromosome aberrations. SWANSON (1949) has previously shown that the posttreatment could be delayed for 18 hours, and that pretreatment could be given as many as 96 hours before X-radiation, with qualitatively the same effect upon chromatid exchange aberrations. It can be seen that the time interval during which infrared is effective in increasing aberrations is of rather long duration. Indeed, the time intervals which have been tried by no means represent limits within which the infrared must be applied. The data in table 2 show that the effectiveness of the treatments does not depend upon the time of delay. That is, there is no substantial decrease in the effect with an increase in the delay. It seems apparent that the actual time limits have not been reached as yet. This is probably true also for the pretreatment data of SWANSON (1949). It must be pointed out, however, that KAUFMANN and WILSON (1949) have reported that there is no effect of posttreatment on

chromosome aberrations induced in Drosophila. Recent, preliminary data of GLASS (1950) indicate that this concept may need revision, since he has found increases induced by infrared posttreatment in both male and female Drosophila.

By smearing the anthers of irradiated buds four days after X-radiation, the metaphase figures scored were those which resulted from chromosomes X-radiated in interphase. SAX (1941) has shown that the interphase chromosomes are single to X-rays, whereas the prophase chromosomes are double. Thus, treatment with X-rays in interphase results in the production of chromosome rather than chromatid aberrations. The changeover from chromatid to chromosome breaks begins at about 26 hours after X-radiation; there then follows a period during which both chromosome and chromatid aberrations are recovered in the same cell, the number of chromatid aberrations recovered decreasing with time; at four days after X-radiation, only chromosomal aberrations are recovered. In the case of the 72- and 96-hour delay series reported in this paper, the treated anthers were smeared at 26 and 0 hours after the infrared treatment. That is, the infrared treatment was given during that period in which the chromosomes are effectively double, and in which chromatid aberrations can be induced by X-rays. At the 96-hour period, the posttreatment must have been given in late prophase or metaphase, since fixation immediately followed the infrared treatment. It is significant that only chromosome aberrations were recovered. Of equal significance is the fact that SWANSON (1949) has shown that infrared pretreatment given during interphase (his 96-hour pretreatment) did not result in the production of chromosome aberrations when the X-ray treatment is given during that period when only chromatid aberrations are induced. *From these pieces of evidence it can be concluded that the type of aberration induced is independent of the time at which the infrared treatments are given, and that the determining factor is the time of X-radiation.*

Further, since those buds from the 96-hour posttreatment series were smeared immediately after the infrared treatment, and since an increase has been observed, the recombination which resulted in an increased yield of detectable aberrations must have taken place during the infrared treatment. The same holds true for the 18-hour delay treatments shown for chromatid breaks by SWANSON (1949). *Therefore, it is evident that the determination of the type of aberration recovered (chromosome or chromatid) is independent of the time of recombination, and dependent solely upon the singleness or doubleness of the chromosomes at the time of X-radiation. When X-radiation is given at interphase, the type of aberration recovered is always chromosomal, whether the breaks recombine at interphase, prophase, or pre-metaphase.* The results of varying the time of infrared posttreatment show this quite clearly. It must be emphasized that the breaks induced in the 72- and 96-hour posttreatment series are recombining during that period when the action of X-radiation, if given then, would be to induce chromatid aberrations. It is difficult, therefore, to see why the additional recombination, taking place

at the time of infrared treatment, does not follow a course determined by the singleness or doubleness of the chromosome; however, there was no evidence of chromatid fusions of any type. Our observations indicate no apparent difference, at the points of recombination, between those chromosomes receiving X-rays and infrared as opposed to those receiving only X-rays. These data consequently point to the conclusion that the breaks induced by X-radiation in the interphase chromosome behave in a manner qualitatively different than those induced in the prophase chromosome. The process of doubling (becoming double to X-rays) does not itself affect the types of aberration resulting through recombination, even though the recombination may occur after the doubling process has obviously taken place.

Evidence of a different nature bearing on this problem has been presented by McCLINTOCK (1942). She has shown in maize that aberrations resulting from breakage and recombination during meiotic divisions are always chromatid aberrations (fusions of sister half-chromatids), whereas the aberrations resulting from breaks which recombine in the interphase nuclei of the zygote are chromosomal. In both cases the breakage was at anaphase of the preceding division. Therefore, it is the time of recombination after breakage, or possibly the type of nucleus in which recombination takes place, and not the time of breakage which seems to be important in maize. The results obtained with infrared cannot be reconciled with this interpretation. Consideration of the mechanism by which infrared radiations exert their effect may shed some light on this subject.

The mechanism of infrared action

All of the data which have so far been gathered on the effects of X-radiation have indicated that only two processes are involved in the production of chromosomal aberrations: the production of breaks and their subsequent recombination. Therefore the data presented here are open to two distinct interpretations. Either the infrared affects the number of primary breaks in the chromosome, or it in some way accelerates the process of recombination so that a larger number of aberrations are recovered from the same number of primary breaks. The third possibility remains that it may do both, of course. It is known that infrared alone will not produce detectable chromosome aberrations in Tradescantia (SWANSON and HOLLAENDER 1946). It is possible that submicroscopic lesions are induced by the infrared treatment and appear as detectable aberrations after the addition of X-radiation. The energy levels of absorbed infrared make this possibility unlikely, however. Also, it has been shown in this study, and by SWANSON (1949), that the type of aberration induced is independent of the time of application of the infrared. If the infrared treatments induce sub-microscopic lesions, the type of aberrations recovered as an added increment should be dependent upon the time at which the infrared treatments were given. Since this is not so, the effect of infrared cannot be considered to be a weakening of discrete loci in the chromosome. On the other hand, it is possible to say that the action of the infra-

red is to cause a general weakening of the entire chromosome framework such that the breakage threshold of the chromosome to high energy radiations is effectively lowered. This postulate covers the pretreatment cases adequately, but to explain the results of posttreatment, it is necessary to consider that the X-radiation is capable of inducing submicroscopic lesions of the chromosome as well as complete breaks. Since the time of X-radiation is the determining factor in the type of breakage recovered (chromosome or chromatid) in Tradescantia, this interpretation fits the data readily; and the action of infrared posttreatments can be considered to have produced a general weakening of the chromosome strands, with the result that those loci previously damaged by X-radiation would undergo complete disunion and give rise to an increase in detectable aberrations.

SWANSON and HOLLAENDER (1946) and SWANSON (1949) have suggested that the action of the infrared is to increase the number of primary breaks of the chromosome. SWANSON (1949) has considered the infrared action on chromatid aberrations with both pre- and posttreatments. From these studies, he has postulated that X-rays are capable of producing both complete and partial lesions, and that infrared is capable of producing only partial lesions. The partial lesions are considered to be incapable of undergoing recombination until they become complete breaks, which can happen only with the addition of supplementary radiation (X-ray or infrared as the case may be). SWANSON has termed these partial lesions "potential breaks" from their postulated behavior. In the light of the data presented here, it seems necessary to revise SWANSON's interpretation. As it has been pointed out above, the action of the infrared can no longer be considered to be on discrete loci. It is suggested, therefore, that the production of "potential breaks" is limited to the X-rays alone. These "potential breaks" are considered to be nondetectable lesions of the chromosome structure, *i.e.*, weakened loci which would not participate in the process of breakage and recombination without the added stimulus from the absorbed infrared.

In considering that X-rays produce a spectrum of alterations in the chromosomes which have varying potentialities in so far as their subsequent behavior is concerned, the proposed hypothesis is in agreement with that of KAUFMANN and WILSON (1949) which was formulated to explain the infrared effects on chromosomal aberrations in Drosophila. The two hypotheses, however, are not in agreement as to the mechanism of action of infrared. Whereas KAUFMANN and WILSON propose that infrared facilitates recombination at the expense of restitution, the number of breaks remaining the same, the present hypothesis suggests, as a more plausible explanation, that the application of infrared leads to an increase in the number of breaks, whether it is given before or after X-radiation. Accordingly, it is proposed that infrared serves to increase the number of breaks by increasing the fragility of the chromosomes toward X-rays. This is supported by the fact that single and isochromatid deletions, as well as exchange aberrations, are increased by

infrared (table 1; SWANSON and HOLLAENDER 1946; SWANSON 1949). Posttreatment, on the other hand, raises weakened, but still intact, loci to the status of open breaks by completing the fracture. Those open breaks already induced by X-rays alone have restituted or recombined, and are unaffected by the subsequent treatment with infrared. The time at which infrared completes the fracture of weakened loci is unimportant, since recombination can take place at any time. This is suggested by the fact that the rate of increase shows no obvious quantitative relationship with delay of infrared treatment after X-radiation (compare 48- and 72-hour delay series, table 2). On this basis, recombination and restitution proceed at a rate determined only by the availability of labile broken ends, the processes themselves being unaffected by infrared. The failure of KAUFMANN and WILSON (1949) to obtain an increased frequency of aberrations in Drosophila with infrared posttreatment is somewhat difficult of explanation, although the preliminary data of GLASS (1950 and unpublished) suggest that posttreatment increases can be achieved.

If, as suggested, the infrared acts primarily to increase the number of breaks capable of recombination with other similarly broken ends, its final effect on X-radiated chromosomes is similar to that resulting from increased oxygen tensions and low temperatures (THODAY and READ 1947; RILEY and GILES 1950; SAX 1947; FABERGÉ 1950). The mechanisms of action must be quite dissimilar, however, for unlike infrared, increased oxygen tensions and low temperatures are effective only during the period of irradiation.

SUMMARY

From the data presented in this paper on the effects of infrared radiations when used as supplementary treatments with X-radiation, it has been possible to conclude the following: 1) pretreatment and posttreatment with infrared radiations have been shown to increase the frequency of chromosome aberrations induced by X-radiation; 2) the posttreatment may be delayed 93–96 hours after X-radiation with no decrease in the effectiveness of the infrared radiations; 3) the type of aberration induced is dependent upon the time at which the X-radiation is administered, and is independent of the time of infrared radiation and the time of recombination.

The various hypotheses which have been advanced to explain the effect of infrared treatment are discussed. The potential break hypothesis is supported by these data. Certain modifications of the original hypothesis as given by SWANSON (1949) are necessary. The implications of this hypothesis are discussed in the light of the data presented.

ACKNOWLEDGMENT

The author would like to express his indebtedness to PROF. CARL P. SWANSON for his helpful criticism and direction during the course of this study.

LITERATURE CITED

FABERGÉ, A. C., 1950 Chromosome breakage by X-rays at low temperature and the radiodecomposition of water. Genetics **35**: 104–105.

GLASS, H. B., 1950 The effects of supplementary treatment with infrared radiation on X-ray-induced lethals and chromosome aberrations in females of *Drosophila melanogaster*. Genetics **35**: 109–110.

KAUFMANN, B. P., ALEXANDER HOLLAENDER and HELEN GAY, 1946 Modification of the frequency of chromosomal rearrangements induced by X-rays in Drosophila. I. The use of near infrared radiation. Genetics **31**: 349–367.

KAUFMANN, B. P., and HELEN GAY, 1947 The influence of X-rays and near infrared rays on recessive lethals in *Drosophila melanogaster*. Proc. nat. Acad. Sci. **33**: 366–372.

KAUFMANN, B. P. and KATHERINE WILSON, 1949 Modification of the frequency of chromosomal rearrangements induced by X-rays in Drosophila. IV. Posttreatment with near infrared radiation. Genetics **34**: 425–436.

LEA, D. C., and D. G. CATCHESIDE, 1942 Induction by radiation of chromosomal aberrations in Tradescantia. J. Genetics **44**: 216–245.

MARINELLI, L. C., B. R. NEBEL, N. GILES and D. R. CHARLES, 1942 Chromosomal effects of low X-ray doses on five-day Tradescantia microspores. Am. J. Bot. **29**: 866–874.

MCCLINTOCK, B., 1941 The stability of broken ends of chromosomes in *Zea mays*. Genetics **26**: 234–282.

1942 The fusion of broken ends of chromosomes following nuclear fusion. Proc. nat. Acad. Sci. **28**: 458–463.

RILEY, H. P., and NORMAN H. GILES, JR., 1950 The effect of oxygen on the frequency of X-ray-induced chromosomal aberration in Tradescantia microspores. Genetics **35**: 131–132.

SAX, K., 1941 Types and frequencies of chromosomal aberrations induced by X-rays. Cold Spr. Harb. Symp. Quart. Biol. **9**: 93–101.

1942 The distribution of X-ray-induced chromosomal aberrations. Proc. nat. Acad. Sci. **28**: 229–233.

1943 The effect of centrifuging upon the production of X-ray-induced chromosomal aberrations. Proc. nat. Acad. Sci. **29**: 18–21.

1947 Temperature effects on X-ray-induced chromosome aberrations. Genetics **32**: 75–78.

SAX, K., and E. V. ENZMANN, 1939 The effect of temperature on X-ray-induced chromosome aberrations. Proc. nat. Acad. Sci. **25**: 397–405.

SWANSON, C. P., 1947 The effect of infrared treatment on the production of X-ray-induced changes in the chromosomes of Tradescantia. Am. J. Bot. **34**: 12a.

1949 Further studies on the effect of infrared radiations on X-ray-induced chromatid aberrations in Tradescantia. Proc. nat. Acad. Sci. **35**: 237–244.

SWANSON, C. P., and ALEXANDER HOLLAENDER, 1946 The frequency of chromatid breaks in Tradescantia as modified by near infrared radiation. Proc. nat. Acad. Sci. **32**: 295–302.

THODAY, J. M., and J. READ, 1947 The effect of oxygen on the frequency of chromosome aberrations produced by X-rays. Nature **160**: 608.

GENE DOSAGE RELATIONS FOR HAPLOIDS AND DIPLOIDS OF HABROBRACON [1]

A. M. CLARK AND C. J. MITCHELL

Department of Biological Sciences, University of Delaware

Received June 12, 1950

GENE action has often been studied by investigating gene dosage relations. This has been done by the addition of chromosome fragments or of entire chromosomes to different genetic backgrounds, exemplified by the work of SCHULTZ (1935) for the gene shaven in Drosophila and of BLAKESLEE (1930) in Datura. STERN and HEIDENTHAL (1944) have shown by their work on position alleles in Drosophila that the effect of gene dosage may also be modified by changing the spatial relationships of genic material. In the above studies, interpretations are based to a certain extent upon changed genic balance conditions. To a limited degree the addition of entire chromosome sets has been used to study gene dosage. Here, the conditions of genic balance appear to remain constant.

In Drosophila, comparison of differences in gene quantity can be made normally only for factors that are sex-linked, since the male is simplex for these genes while the female is duplex. It has been shown, in general, that the gene in single quantity in the male produces an effect equal to that of the doubled gene condition found in the female. STERN (1929) and MULLER (1932, 1948) postulate a dosage compensation due principally to naturally selected intra-chromosomal modifiers. MULLER (1948) has presented evidence which indicates that the mechanism of dosage compensation holds for genes located in the X-chromosome of *Drosophila melanogaster*, but not for genes located in the autosomes since here males and females normally have the same dosage.

It appears that Habrobracon may offer favorable experimental conditions for the study of gene dosage relations since haploids and diploids normally occur within this organism. It would be interesting to determine if under these conditions of ploidy a system of compensating genes has been built up and also whether most of the mutant genes are hypomorphic such as those found in Drosophila. Three sex types exist in Habrobracon; haploid males arising from unfertilized eggs, diploid males and diploid females arising from fertilized eggs. Thus, comparison of mutant genes can be made between individuals of the same sex differing in the number of chromosome sets (haploid males and diploid males), as well as between individuals of different sexes which have the same number of chromosome sets (diploid males and diploid females).

Most of the work on the effects of extra chromosome sets on the morphology and physiology of organisms has been done on plants, where polyploidy

[1] This investigation was aided by a grant from the Committee on Research, UNIVERSITY OF DELAWARE.

can be readily induced. Little of this work has been done on animals since it is generally held that viable polyploids are not so readily obtained. For the amphibians, methods for the induction of polyploids have been developed (FANKHAUSER 1945), but these organisms have not been studied along genetic lines. Since comparison of phenotypic differences between diploids and polyploids are usually made with wild-type individuals, it is not possible to separate the effects due to increased gene dosage from those due to an increase in chromatin mass. In Habrobracon, individuals differing in the number of chromosome sets occur normally. Also, many mutant types have been isolated and bred (WHITING 1932, 1934). These mutant types affect eye color, eye size and shape, wing length, wing venation, antennal flagellar length and width. The antennae are particularly versatile, showing many types of inherited modification. It seems, then, that Habrobracon should be useful not only for genetic studies but also for the study of physiological and cytochemical differences between individuals differing in ploidy.

Organisms in which the number of sets of chromosomes has been changed usually show differences in cell and nuclear size. DOBZHANSKY (1929) has shown for Drosophila, that a single microchaeta corresponds to an epithelial cell making up the wings. This has also been observed for Habrobracon (SPEICHER 1935). It is possible, therefore, to obtain measurements of cell size by means of microchaetal counts. Comparison of wing microchaetae in Habrobracon shows that diploid male wing cells are larger than diploid female wing cells, which in turn are larger than the wing cells of the haploid males (SPEICHER 1935; RISMAN 1941; GROSCH 1945). Diploid males have larger fat cells than haploid males and these are larger than the fat cells of the diploid females (GROSCH 1948; CLARK and GROSCH 1948). Determination of cell and nuclear areas for the gut epithelium of adults (GROSCH and CLARK 1949) shows that the cell and nuclear size is identical for the diploid males and females but is smaller for the haploid males and that the nuclear-cytoplasmic ratio is the same for all three types. Thus, differences in size relationships hold for different tissues. Diploid males do not occur as often among offspring as might be expected on the basis of a random fertilization and are thought, therefore, to be less viable than the other two classes.

Studies of haploid-diploid relations in Habrobracon have been made primarily to study the important problems of sex determination and evolution (WHITING 1943a, 1945). WHITING (1943b), in his studies on androgenesis noted that homozygous fused diploid males have shorter antennae than their fused haploid brothers. He regards this as being due to the doubled chromosome number. CLARK and MITCHELL (1948) noted that homozygous stubby diploid males have shorter antennae than the stubby haploid males. These observations indicated that a more detailed investigation of gene dosage relations among haploids and diploids might be fruitful.

MATERIALS AND METHODS

Sex in Habrobracon has been found to be determined by a series of multiple sex alleles, *xa, xb, xc,* etc. (WHITING 1943a). Haploid males arise from

unfertilized eggs and have any one of these alleles (*xa, xb, xc,* etc.). Diploid males and diploid females arise from fertilized eggs. The female is heterozygous (*xa/xb, xa/xc, xb/xc,* etc.) while the diploid male is homozygous (*xa/xa, xb/xb, xc/xc,* etc.). Accordingly, diploid males are obtained from 2-allele crosses such as *xa/xb* by *xb,* but not from 3-allele crosses such as *xa/xb* by *xc.* Thus, in order to obtain diploid males it was necessary to synthesize 2-allele stocks. Number 25 stock was used as a base stock because a higher incidence of diploid males occurs in it than in the other available inbred stocks (GROSCH 1945).

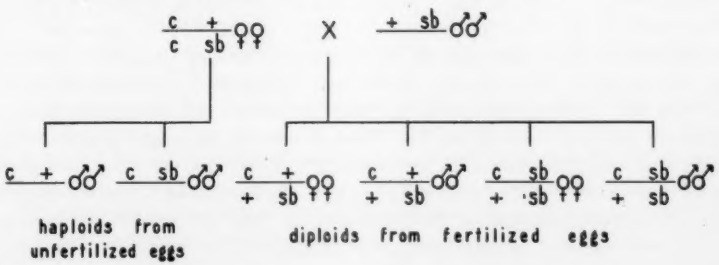
Since, in the wild-type, haploid males cannot readily be distinguished from their diploid brothers, a recessive marker gene was used. Thus female homozygous for the recessive marker gene cantaloup (eye color) were mated to black-eyed haploid males. From this cross the unfertilized eggs result in cantaloup-eyed haploid males while the fertilized eggs give rise to black-eyed diploid offspring (male and female) which are heterozygous for eye color.

The breeding procedure was as follows: A haploid male carrying the mutant to be tested was crossed to a cantaloup-eyed female from stock 25. An F_1 virgin female (heterozygous for the introduced mutant and the eye color) was selected and allowed to lay eggs. From these eggs, F_2 haploid males were obtained. A cantaloup-eyed mutant male was crossed to his mother. From this cross a 2-allele stock was established which contained the mutant to be studied as well as the marker gene. All subsequent offspring were derived from this cross.

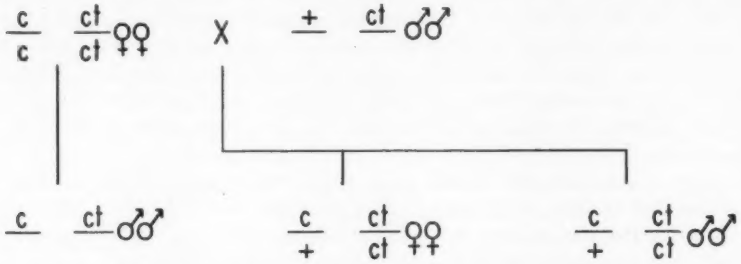
The breeding procedure used in the derivation of stocks does not insure that these stocks are homozygous for the residual genotype. However, all of the mutants were bred into the same base stock (No. 25c) and all of the progeny used in each of the final crosses were derived from a single mother to son mating. The nature of such crosses, in which diploid and haploid individuals are obtained, insures that the different classes of animals are genetically equivalent in modifiers.

In some of the mutants studied, comparisons were made on heterozygous as well as homozygous individuals. For other mutants, only homozygotes were studied.

For comparison of heterozygotes, six classes of offspring were obtained. For example: *stubby* (*sb*)



For comparison of homozygotes, three classes of offspring were obtained. For example: *cut* (*ct*)



Females were placed in shell vials with Ephestia larvae which they parasitize and upon which they lay eggs. Transfer of females to vials with fresh Ephestia was made every four days. All wasps were reared at 30°C.

Individuals of similar body size were preserved in an alcohol-glycerine mixture. The structures to be studied were subsequently removed, washed in xylol and mounted on slides with balsam. Measurements were made directly from slides by means of an ocular micrometer or from camera lucida drawings.

THE WILD-TYPE PHENOTYPE OF HAPLOIDS AND DIPLOIDS

Haploids and diploids of the wild phenotype were compared in order to determine similarities and differences among them and the magnitude of these differences. This information was then used to evaluate the extent of the differences between haploid and diploid mutant types, and thereby determine whether the diploid mutants show a difference in phenotype.

Stock No. 25c females were crossed to No. 25+ males. Three classes of offspring were obtained: haploid males with cantaloup-eye color, diploid males with black eye-color, and diploid females with black eye-color. Fifty offspring of similar body lengths (2.65–2.75 mm) were selected from each class. The right primary wings, right secondary wings, and right antennae were removed and mounted.

Habrobracon females normally have shorter antennae and fewer antennal segments than do males (fig. 1). This represents a secondary sex character in the wild-type state. Although haploid males and diploid males are similar in appearance, statistical differences do exist. GROSCH (1945), in a study of structural differences among haploids and diploids in wild-type stocks, showed that the antennal flagella length is the same for haploid and diploid males, but that the haploids had more flagellar segments and, therefore, shorter segments. He also showed that the haploid males have longer flagellar segments than females. The data reported here (table 1) are in accord with these observations. The measurements for segment length were obtained from the seventh segment of the males and the fifth segment of the females. These were selected because they were middle segments of the flagellum,

TABLE 1

Mean measurements of wild-type offspring from 25c ♀ × 25 + ♂.*

	$\frac{c}{-} \pm ♂$	$\frac{c}{+} \frac{+}{+} ♂$	$\frac{c}{+} \frac{+}{+} ♀$
Primary wing length (mm)	2.262 ± 0.012	2.298 ± 0.012	2.395 ± 0.013
Secondary wing length (mm)	1.958 ± 0.012	1.960 ± 0.013	2.051 ± 0.007
Secondary wing area (mm)	0.772 ± 0.008	0.765 ± 0.008	0.825 ± 0.010
Number of michochaetae per 0.01 mm² of wing surface in radial cell	15.36 ± 0.16	10.41 ± 0.12	13.73 ± 0.14
Antennal flagellar length (mm)	1.8634 ± 0.0149	1.8359 ± 0.0120	1.1080 ± 0.0081
Segment length[a] (mm)	0.0988 ± 0.0009	0.1026 ± 0.0008	0.0902 ± 0.0007
Segment width[a] (mm)	0.0722 ± 0.0004	0.0641 ± 0.0008	0.0765 ± 0.0004

* Measurements are based upon 50 individuals per group.

[a] Measurements made of the 7th segment for the males and of the 5th segment for the females.

not in the tapered part of the normal antennae. Comparison of segment width among these three types shows that the females (0.0765 ± 0.0004 mm) are wider than the haploid males (0.0722 ± 0.0004 mm), and the haploid males are wider than the diploid males (0.0641 ± 0.0008 mm). Correlation of segment width with segment length shows an inverse relationship. The diploid males have longer and thinner segments while the females have shorter and thicker segments. On this basis it is assumed that each segment has the same volume and that an increase in length is compensated for by a decrease in width.

Comparison of primary wing lengths (table 1) for the diploid males (2.298 ± 0.012 mm) and the haploid males (2.262 ± 0.012 mm) shows that the difference is on the borderline of statistical significance. The primary wing length for the females (2.395 ± 0.013 mm) is significantly greater than the other two groups. Differences in secondary wing length between haploid males (1.958 ± 0.013 mm) and diploid males (1.960 ± 0.013 mm) are non-significant, but both of these groups have wings that are significantly shorter than the wings of the females (2.051 ± 0.007 mm). The females, then, have longer primary and secondary wings than the males. Measurements of wing areas for the secondary wings show that the females (0.825 ± 0.010 mm²) are significantly greater than the diploid males (0.765 ± 0.008 mm²) and the haploid males (0.772 ± 0.008 mm²), with the latter two groups being statistically similar. The method used in estimating statistical significance was the standard error of the difference.

Microchaetal counts in the radial cell of the primary wing show that the average wing cell size for these groups decreases in the order of diploid male, diploid female, and haploid males (table 1). These observations are in accord with the findings of SPEICHER (1934) and GROSCH (1945).

The foregoing shows that there is an overlapping of phenotype between the wild-type haploid and the diploid males with no extreme structural differ-

ences. Therefore, the doubled chromosomal complement found in the diploid male produced no marked qualitative or quantitative differences in gross structure.

THE MUTANT PHENOTYPES OF HAPLOIDS AND DIPLOIDS

Stubby (sb), Fused (fu), Coalescent (co)

The mutants, stubby, fused and coalescent, affect antennal flagellar length. In all of these mutant types the flagella are shorter than the wild type. This shortening is accompanied by varying degrees of fusion of the segments, apparently due to the inability of the antennae to differentiate properly into flagellar segments. Since antennal flagellar length is a secondary sex character, the mutant type females have shorter flagella than their corresponding mutant type males (Plate 1).

Fused—Fusion of flagellar segments is so complete that the flagellum appears unsegmented. There is also a fusion of the segments of the tarsus into a single segment, and the maxillary palpi and the labial palpi are each fused into single segments.

Stubby—Varying degrees of fusion and aberrant articulation of flagellar segments occur. These are never as extreme as in fused. There is no effect upon the palpi and the tarsi.

Coalescent—Varying degrees of fusion and aberrant articulation of flagellar segments occur. There is an overlapping with the stubby phenotype. Segments making up the palpi show aberrant articulation. The normally occurring 5-jointed tarsus is not found for many coalescent individuals; tarsal segments may be split transversally into two, thereby giving the appearance of additional segments.

Comparison of antennal flagellar lengths between the haploid and diploid males for each mutant type (stubby, fused and coalescent) shows that the diploid male flagellum is shorter (Plate 1). However, there is some overlapping since a few of the diploid male antennae are as long as the mutant haploid male antennae, while others are as short as the shortest female antennae.

For the mutant type stubby, sufficient diploid males were obtained for statistical treatment (table 2). The haploid stubby males have a mean flagellar length of 0.8562 ± 0.0164 mm as compared with 0.5399 ± 0.0174 mm for

TABLE 2

Mean antennal flagellar length (mm) of offspring from $\frac{c\ +}{c\ sb}$ ♀ × $\frac{+\ sb}{}$ ♂.

	$\frac{c\ +}{}$ ♂	$\frac{c\ sb}{}$ ♂	$\frac{c\ +}{+\ sb}$ ♂	$\frac{c\ sb}{+\ sb}$ ♂	$\frac{c\ +}{+\ sb}$ ♀	$\frac{c\ sb}{+\ sb}$ ♀
Number of flagella measured	68	69	72	59	70	72
Antennal flagellar length	1.8571 ± 0.0154	0.8562 ± 0.0164	1.7360 ± 0.0127	0.5399 ± 0.0174	1.1222 ± 0.0074	0.4394 ± 0.0071

the homozygous diploid stubby males. The difference between these means is significant. The shorter antennal flagella found for the homozygous diploid, when compared with the haploid males, indicates greater gene dosage effects for the diploid males.

Comparison of the homozygous diploid stubby male flagella (0.5399 ± 0.0174 mm) with those of the homozygous diploid stubby females (0.4394 ± 0.0071 mm) shows that while the males approach the females in antennal flagellar length, the difference between the means is significant. However, since the females normally have shorter antennae than the males, direct comparisons of flagellar length for males and females may not suffice for an accurate determination of quantitative genic differences.

There is a difference in expressivity between diploid males and females heterozygous for stubby. Examination of the segments making up the flagellum of the antenna shows a certain amount of aberrant articulation for some of these heterozygous individuals. For the heterozygous diploid males, 24 out of 72 showed aberrant articulation while for the heterozygous females only 3 out of 70 show this trait. For the females this aberrant articulation was restricted to the distal segments while in the males it was found also for middle segments. The expressivity was also greater for the diploid males than the females. Essentially the same condition is found when comparing males and females heterozygous for the mutant type fused. Thus, examination of heterozygotes shows that stubby and fused are semi-dominant in the diploid male state, but are masked by the wild-type allele in the diploid female state.

In the preceding section the data indicate that the mutants stubby, fused and coalescent show marked differences in phenotype between the haploid and diploid males. For other mutants reported below, obvious differences were not found. In the study of some of these mutants it was discovered that certain of the characters do not lend themselves to quantitative analysis or are influenced by sex or male diploidy.

Thin (th)

This is an antennal mutant in which the flagellar segments are thinner than wild-type. Comparison between wild-type and thin phenotypes of haploid males, diploid males, and diploid females show that thin individuals have shorter flagella (table 3). The mean segment length is also shorter. The effect of male diploidy upon segment length is similar here as in wild-type, in that the diploid males have longer segments than the haploid males or females. There is no effect upon segment number. Comparison of segment widths between $\underline{c\ th}$ males and $\frac{c\ th}{+\ th}$ males shows that the homozygous diploid males have thinner antennae. However, this difference in structural dimension between these groups, although statistically significant, is not sufficiently great to be explained in terms of enhanced gene dosage. It seems better to attribute this difference to the effects of male diploidy, since differences of a similar magnitude are found in the wild-type.

TABLE 3

Antennal flagellar measurements of offspring from $\frac{c}{c}\ \frac{+}{th}$ ♀ × $\frac{+}{+}\ \frac{th}{th}$ ♂.

	$\frac{c}{+}$ ♂	$\frac{c}{+}\ \frac{th}{th}$ ♂	$\frac{c}{+}\ \frac{+}{th}$ ♂	$\frac{c}{+}\ \frac{th}{th}$ ♂	$\frac{c}{+}\ \frac{+}{th}$ ♀	$\frac{c}{+}\ \frac{th}{th}$ ♀
Number measured	62	71	52	38	49	40
Flagellar length mm	1.824 ± .012	1.621 ± .014	1.789 ± .024	1.552 ± .023	1.040 ± .010	0.928 ± 0.014
Number flagellar segments	19.45 ± .09	19.35 ± .09	17.54 ± .14	17.39 ± .18	12.33 ± .07	12.52 ± .10
Segment length mm	.0877 ± .0009	.0777 ± .0012	.0992 ± .0007	.0899 ± .0012	.0865 ± .0006	.0763 ± .0011
Segment width mm	.0736 ± .0004	.0495 ± .0005	.0664 ± .0005	.0445 ± .0008	.0763 ± .0005	.0534 ± .0005

Long (l)

This is an antennal mutant in which each flagellar segment is longer than the wild-type, thereby resulting in longer flagella. Comparison between wild-type and long phenotypes of haploid males, of diploid males and of diploid females shows that the long mutant types have longer antennae than the corresponding wild type (table 4). In each case the mean segment length is also longer. There is no apparent effect upon segment number. Comparison of mean segment length shows that the homozygous long diploid males exceed dimensions for the long haploid males, and the heterozygous long diploid males exceed dimensions for the wild-type haploid males. These differences are not sufficiently large to attribute them to enhanced gene dosage. The increased segment length, therefore, seems to be due to the effects of male diploidy.

For the antennal mutants thin and long, statistically significant differences between diploid males and haploid males were obtained. However, these differences were of the same order of magnitude as those found between the wild-type haploid and diploid males. These differences, therefore, seem better

TABLE 4

Antennal flagellar measurements of offspring from $\frac{c}{c}\ \frac{+}{l}$ ♀ × $\frac{+}{+}\ \frac{l}{l}$ ♂

	$\frac{c}{+}$ ♂	$\frac{c}{+}\ \frac{l}{l}$ ♂	$\frac{c}{+}\ \frac{+}{l}$ ♂	$\frac{c}{+}\ \frac{l}{l}$ ♂	$\frac{c}{+}\ \frac{+}{l}$ ♀	$\frac{c}{+}\ \frac{l}{l}$ ♀
Number measured	26	24	24	25	25	25
Flagellar length mm	1.9699 ± .0431	2.3759 ± .0114	1.9112 ± .0164	2.1053 ± .0300	1.2681 ± .0202	1.4471 ± .0185
Number flagellar segments	19.69 ± .18	19.79 ± .18	17.07 ± .16	16.91 ± .20	12.60 ± .16	12.47 ± .03
Segment length mm	.1000 ± .0011	.1202 ± .0009	.1113 ± .0016	.1244 ± .0015	.1006 ± .0009	.1174 ± .0012

explained as due to the effects of male diploidy rather than to enhanced dosage of these genes in the diploid state.

Kidney-Heidenthal (K^h)

This phenotype is distinguished from wild-type by the presence of a smaller head. The compound eyes are also smaller and sometimes do not appear. Examination of K^h haploids and diploids failed to reveal any noticeable difference in phenotype expression.

Bulge (*Bu*)

The mutant type bulge is recognizable by the marked transverse protrusion of the eyes. Examination of bulge haploid males, homozygous bulge diploid males, and homozygous bulge diploid females did not reveal noticeable differences among these classes.

Small-wings (*sw*)

This phenotype is characterized grossly by the presence of shorter primary and secondary wings. Lengths of primary and secondary wings were determined for $\underline{c+}$ males, $\underline{c\ sw}$ males, $\frac{c\ +}{+\ sw}$ males, $\frac{c\ sw}{+\ sw}$ males, $\frac{c\ +}{+\ sw}$ females, and $\frac{c\ sw}{+\ sw}$ females obtained from the cross $\frac{c\ +}{c\ sw}$ females $\times +\ sw$ males. Differences in wing length between the haploid and diploid mutants were not sufficiently great to be interpreted as clear cut dosage differences. Homozygous diploid male wings were longer than were the small wing haploid males, but this condition also exists in the wild-type.

Reduced (*r*)

The reduced mutant type shows shorter primary and secondary wings than the wild-type and also the absence of the medio-cubital crossvein and a certain amount of vein disorganization. Comparison of haploid males and diploid males showed no apparent difference in phenotype, either in wing length or vein disorganization.

Cut (*ct*)

The mutant cut affects primary and secondary wings. For the primary wing the margin from the stigma to the apex is straightened instead of being rounded as is normally found for the wild-type. The secondary wings show varying degrees of scalloping recognizable by a decrease in wing area size and the absence of bristles at the margin of scalloped regions. Cut is variable in expression in both the secondary and primary wings. There is, however, a certain degree of correlation between the amount of scalloping in the secondary wing and wing area.

Comparison of mean areas for the secondary wings shows that the diploid male wings are significantly smaller than those of the haploid males or diploid females (table 5). Since differences in secondary wing areas for the wild-type

TABLE 5

Mean areas for secondary wings of offspring from $\frac{c}{c}\ \frac{ct}{ct}$ ♀ × $\frac{+}{+}\ \frac{ct}{ct}$ ♂

	$\frac{c}{+}\ \frac{ct}{ct}$ ♂	$\frac{c}{+}\ \frac{ct}{ct}$ ♂	$\frac{c}{+}\ \frac{ct}{ct}$ ♀
Number measured	115	77	86
Wing area mm²	0.594 ± .008	0.517 ± .010	0.584 ± .012

haploid and diploid males are non-significant, it appears that in cut diploid males the doubled gene content enhances the dosage effect upon the secondary wing. Cut diploid females do not differ from cut haploid males with respect to secondary wing areas. However, wild-type females have a larger secondary wing area than do wild-type haploid males. Thus, the phenotype cut is more strongly expressed in the female than in the haploid male. These data do not indicate whether or not a difference in gene dosage between diploid males and diploid females exists.

DISCUSSION

Studies of gene dosage relations in organisms differing in the number of chromosome sets seem to offer opportunity for the analysis of gene action. Such studies differ from work in which entire chromosomes or chromosome fragments are added to the genetic complex in that the addition of an entire genome would not be expected to alter the conditions of genic balance. Comparison of diploids and polyploids has shown that quantitative differences exist, however, in some cases, qualitative differences have also been shown. In these studies differences in phenotype are usually attributed to the larger cell and nuclear size in polyploids. It is difficult to determine how much of the developmental process can be credited to the changed physical relationships due to the increase in the mass of chromatin material, and how much to the changed chemical relationships due to a differential action of individual genes in the polyploid state.

In the present study, an attempt is made partially to separate the effects of individual genes from an increase in chromatin mass by comparing haploids and diploids in the wild-type state and by the substitution of mutants into these systems. It is never possible to effect completely such a separation since the mutant genes in the diploid are operating in a different genetic system than those in the haploid.

A comparison of wild-type haploid and diploid males shows that there are no distinctive structural differences which can be used to separate them. Differences do exist, but these are of a statistical nature. As a group, diploid males have larger wing cells and fewer, longer, and thinner antennal flagellar segments than haploid males. Thus, an increase from one to two sets of chromosomes results in no striking phenotypic differences. It would appear that haploid and diploid mutant types would contain the same mass of chro-

matin as their comparable wild-types and would differ only in a single gene locus. Therefore, marked phenotypic differences above those shown in the wild-type could be attributed to a change at a single gene locus.

Dosage relations for ten mutant types are reported, involving antennal, head or wing structure. Study of these mutants indicates that no generalization can be made for doubled gene dosages in Habrobracon, since some show increased phenotypic effect while others do not. These data are in accord with the views of GOLDSCHMIDT (1938) who, in considering dosage effects due to the addition of entire chromosome sets, states "The threshold for the maximum effect of gene doses may be different for different genes; therefore, at higher dosage some may increase their effect proportionately, others not (when their threshold is reached)." Thus, the conditions of genic balance would not appear to hold for some of these genes. The homozygous stubby, fused and coalescent diploid males have markedly shorter antennae than their corresponding mutant haploid brothers. This indicates that for these mutants the doubled gene condition results in an enhanced effect upon phenotype. In both the haploid and diploid males the ratio of gene to background is identical, since a doubling of any particular gene is accompanied by a doubling of all the genes in the system. According to the theory of genic balance a resulting phenotype is effected by plus and minus modifiers present throughout all of the chromosomes. These conditions for genic balance do not appear to hold for the mutants stubby, fused and coalescent of Habrobracon.

For the ten mutant types reported here, the most marked differences were obtained for those mutants having a similar phenotype. The mutant types stubby, fused and coalescent have shorter antennae and show fusion of antennal segments. This expression is increased in the diploid male. This indicates that the doubled gene dosage has no meaning in itself but only in so far as these genes are able to act during particular stages of development. Certain developmental processes may be considered more susceptible to doubled gene quantity than others. GOLDSCHMIDT (1938), in attempting to explain phenotypic differences between males and females comparable in genetic constitution for the specified gene loci, has postulated that these genes are operating in different genetic systems. It seems that such an explanation could be used here.

In Habrobracon, haploid males, diploid males and diploid females may differ during certain periods of development and not during others. Genes operating during the developmental periods which differ among these three groups would act differentially, while genes acting during developmental periods which did not differ would show no such differential effect. Pertinent to this is the observation (unpublished) that irradiation of diploid male larvae in cocoons, at a period 72–84 hours after oviposition, results in a shortening of antennae while similar treatment of comparable haploid males and diploid females does not. There is apparently a difference in the sensitive period among these groups. There is also an indication of changed dosage relationships when comparing males and females heterozygous for stubby or fused.

Almost all of the heterozygous females are of wild-type phenotype, while about 30 percent of the heterozygous males show a certain amount of antennal fusion. Thus, the mutants stubby and fused are masked by their wild-type alleles in the female, but are semi-dominant in the male.

SUMMARY

1. Gene dosage effects for haploid males, diploid males, and diploid females were investigated in the parasitic wasp Habrobracon. Determinations were made of phenotypic differences for the wild-type and for ten mutant types. An attempt is made to separate partially the effect of enhanced gene dosage from increased chromatin mass upon the phenotype.

2. Stubby, fused and coalescent, mutant types which are recognizable by antennal shortening and aberrant articulation of antennal segments, are more extreme in the diploid males than in the haploid males. Thus, the conditions of genic balance do not seem to hold for these mutants.

3. The heterozygous stubby and heterozygous fused diploid males show a certain degree of antennal fusion (about 30 percent penetrance). Few heterozygous stubby or fused females show this fusion. These genes, then, are masked by their wild-type alleles in the female state, but are semi-dominant in the diploid male state.

4. The mutant type cut shows greater effect upon scalloping of the secondary wing in the diploid male and diploid female than in the haploid male.

5. The mutant types long, thin, kidney-Heidenthal, bulge, small wing, and reduced show no marked phenotypic differences between haploid males, diploid males, and diploid females. These genes in doubled dose seem to have no greater effect upon phenotype than when present in single dose.

6. The data show that some genes in doubled quantity have an enhanced effect while others do not. Of the ten mutants studied, the three showing the most marked effects were those affecting the same structure in the same way.

PLATE 1

Drawings of antennae for wild-type, stubby, fused, and coalescent in haploid males, diploid males, and diploid females.

Figure 1.—Wild-type haploid male.
Figure 2.—Wild-type diploid male.
Figure 3.—Wild-type diploid female.
Figures 4–5.—Stubby haploid male.
Figures 6–8.—Homozygous stubby diploid male.
Figures 9–10.—Homozygous stubby diploid female.
Figure 11.—Heterozygous stubby diploid male.
Figures 12–13.—Coalescent haploid male.
Figures 14–15.—Homozygous coalescent diploid female.
Figures 16–17.—Homozygous coalescent diploid male.
Figures 18–20.—Fused haploid male.
Figures 21–23.—Homozygous fused diploid male.
Figures 24–25.—Homozygous fused diploid female.

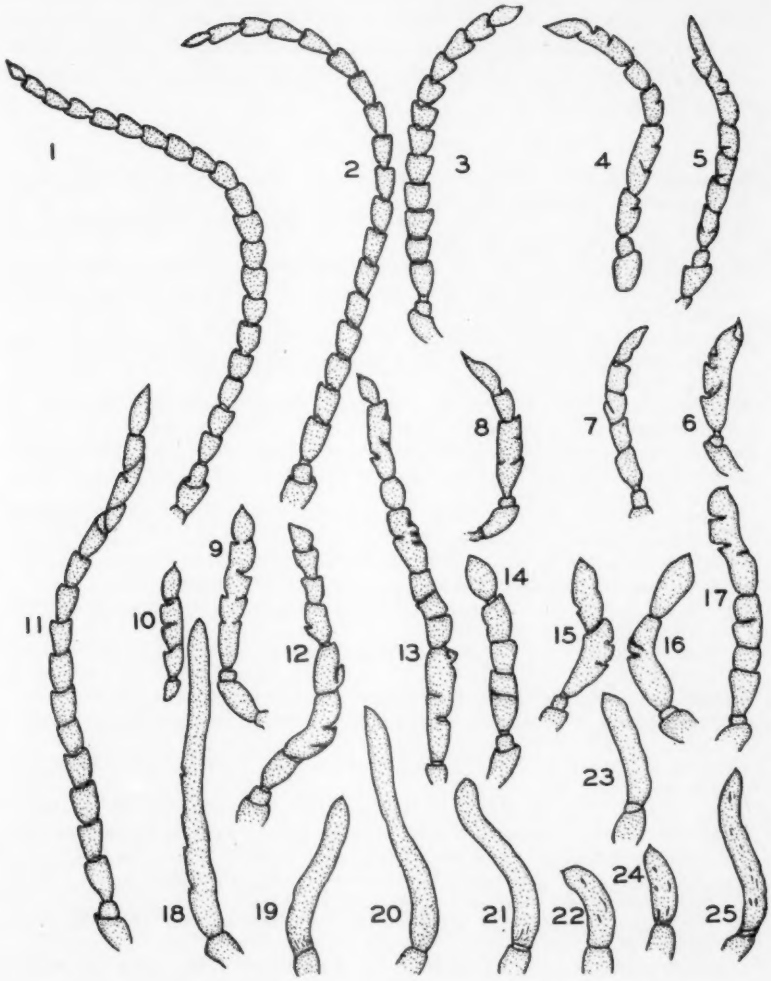


PLATE I

ACKNOWLEDGMENTS

The authors wish to thank DR. P. W. WHITING of the UNIVERSITY OF PENNSYLVANIA and DR. D. S. GROSCH, NORTH CAROLINA STATE COLLEGE, for their suggestions and criticisms during the preparation of this manuscript. The help of MRS. JEAN WILSON, who prepared the drawings, is also acknowledged with appreciation.

LITERATURE CITED

BLAKESLEE, A. F., 1930 Extra chromosomes a source of variations in the Jimson weed. Smithsonian Inst. **3096:** 431–450.

CLARK, A. M., and D. S. GROSCH, 1948 Fat cell size in the mutant small-wing of Habrobracon. Biol. Bull. **95:** 264.

CLARK, A. M., and C. J. MITCHELL, 1948 Dosage relations of haploids and diploids for the mutant stubby of Habrobracon. Rec. Genet. Soc. Amer. **17:** 34, and Genetics **33:** 606–607.

DOBZHANSKY, TH., 1929 The influence of the quantity and quality of chromosomal material on the size of cells in *Drosophila melanogaster*. Arch. Entw-Mech. Org. **115:** 363–379.

FANKHAUSER, G., 1945 The effects of changes in chromosome number on amphibian development. Quart. Rev. Biol. **20:** 20–78.

GOLDSCHMIDT, R., 1938 Physiological Genetics, vi + 375 pp. New York: McGraw-Hill.

GROSCH, D. S., 1945 The relation of cell size and organ size to mortality in Habrobracon. Growth **9:** 1–17.

1948 The cells of the fat body in haploid and diploid types of Habrobracon. J. Elisha Mitchell Sci. Soc. **64:** 178.

GROSCH, D. S., and A. M. CLARK, 1949 Cytological investigations of the gut epithelium in haploids and diploids of Habrobracon. Biol. Bull. **97:** 237–238.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations. Proc. 6th Int. Cong. Genet. **1:** 213–255.

1948 Evidence of the precision of genetic adaptation. The Harvey Lectures, series **43:** 165–229.

RISMAN, G. C., 1941 Cell size in Habrobracon. Rec. Genet. Soc. Amer. **10:** 166.

SCHULTZ, J, 1935 Aspects of the relation between genes and development in Drosophila. Amer. Nat. **69:** 30–54.

SPEICHER, B. R., 1935 Cell size and chromosomal types in Habrobracon. Amer. Nat. **69:** 79.

STERN, C., 1929 Über die additive Wirkung multipler Allele. Biol. Zbl. **49:** 261–290.

STERN, C. C., and G. HEIDENTHAL, 1944 Material for the study of the position effect of normal and mutant genes. Proc. nat. Acad. Sci. **30:** 197–205.

WHITING, P. W., 1932 Mutants in Habrobracon. Genetics **17:** 1–30.

1934 Mutants in Habrobracon. II. Genetics **19:** 268–291.

1943a Multiple alleles in complementary sex determination of Habrobracon. Genetics **28:** 365–382.

1943b Androgenesis in the parasitic wasp Habrobracon. J. Hered. **34:** 355–366.

1945 The evolution of male haploidy. Quart. Rev. Biol. **20:** 231–260.

A GENE THAT CAUSES NATURAL DEATH IN NEUROSPORA CRASSA[1]

T. C. SHENG[2]

Department of Zoology, Columbia University, New York

Received June 19, 1950

NEUROSPORA genetics has contributed much to our understanding of genic action and biosynthesis through the study of mutants that are deficient in their ability to synthesize vitamins, amino acids and other metabolites. These mutants are reparable in the sense that they can be cultured on media supplemented with the required growth factors or metabolites. There are, however, mutants that will not grow or that will grow only slowly at one temperature or another even on complete medium (HOROWITZ 1950). They may be referred to as irreparable mutants (HOROWITZ 1948).

These mutants are of special interest for the following reasons. Firstly, they might be mutants involving multiple-functioned genes (DELBRUCK's discussion following paper by BONNER 1946; HOROWITZ 1948). Secondly, as yet unidentified growth factors might be detected through the study of these mutants. Thirdly, they might be deficient or defective in enzymes not functional in synthetic processes. Most morphological mutants could be of this nature. The irreparable mutant to be described in this paper seems to belong to still another category. It is characterized on the one hand by a normal growth on minimal medium in the young condition and on the other hand by an ever-decreasing growth potential under any condition tested thus far. Besides being irreparable it is therefore of interest in another respect: the vegetative growth from a single ascospore undergoes a process of ageing and natural death. Natural death is defined arbitrarily as the progressive irreversible cessation of growth due to intrinsic causes.

MATERIALS AND METHODS

This mutant, called natural death or *nd*, was obtained after ultraviolet light irradiation of conidia of an albino strain 15300(al_2)*A*. It was selected because it stopped growing as a tiny colony on sorbose plate at 37.8°C but grew larger after the plate was transferred to 25°C.

It could be crossed with all non-*nd* strains tested. Non-*nd* vs. *nd* phenotype of isolated spores was determined by testing in growth tubes at 37.8°C or 32°C. A typical segregation is shown in figure 1. Successive subcultures

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, COLUMBIA UNIVERSITY. This work was done during the tenure of a PUBLIC HEALTH SERVICE FELLOWSHIP sponsored by the NATIONAL CANCER INSTITUTE.

[2] Present address Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California.

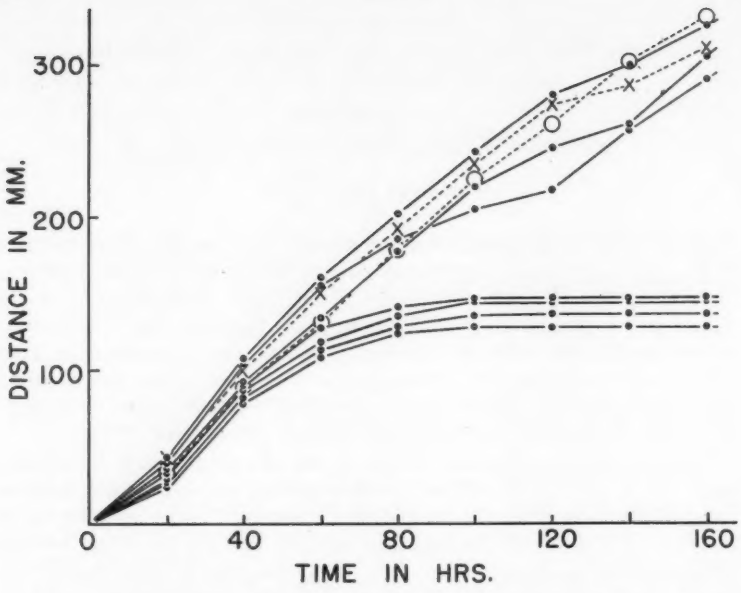


FIGURE 1.—Segregation of *nd* vs. non-*nd* character in an ascus from a cross of *nd al₂ A* (3rd transfer of the original mutant culture) to $+^{nd}$ *al₂ a*–15300. Temperature 37.8°C. o = segregants; × = $+^{nd}$ *al₂ A*–15300; ○ = $+^{nd}$ *al₂ a*–15300.

on slants of the original culture were labelled as the 1st, 2nd, etc. stock transfers and were kept in a refrigerator. All *nd* cultures ceased to grow at the fourth to sixth transfer on slants at 25°C.

The symbols for the mutant genes used in this investigation are as follows: pantothenicless 5531–22 (*pan*), lysineless 4545 (lys_1), albino 4637 (al_1), albino 15300 (al_2), leucineless 33757 (leu_1), p-aminobenzoicless 1633 (*pab*), amycelial 422 (*am*), microconidiating (pe^m), fluffy (*fl*) and natural death (*nd*).

When growth was measured in liquid cultures, 40 ml of Fries No. 3 medium with 1 percent sucrose per 125-ml Erlenmeyer flask was generally adopted. The growth tube method of measuring growth gives a more accurate measurement of instantaneous growth rate and helps one to visualize the limitation of growth and hence was used in most cases (RYAN, BEADLE and TATUM 1943). For measurement of maximal amount of uninterrupted growth at temperatures lower than 32°C, tubes of 62 and 88 cm in length were necessary, because for some unknown reason the total amount of growth was much less if growth was interrupted by subculturing. Short (40 cm) growth tubes with several side arms were used whenever sampling along the tube was desired.

The sorbose plating technique was used whenever discrete colonies were desired (TATUM, BARRATT and CUTTER 1949). Agar with 0.5 percent sorbose plus 0.01 percent sucrose was found to be satisfactory for counting purpose. In attempts to isolate homocaryotic strains, 0.05 percent sorbose plus 0.01 percent sucrose was found to be more suitable.

All test cultures were run in duplicate unless otherwise mentioned.

EXPERIMENTAL RESULTS

Segregation and localization of the nd gene

When an *nd* culture was crossed to a non-*nd* culture a one to one segregation for the *nd* character was observed in each case. In randomly isolated spores a statistical one to one segregation was also observed in each cross.

TABLE 1

The estimation of map distances.

Map distance between	Source of data: Randomly isolated spores (172)			Source of data: Whole asci (24)	Weighted mean	Values from H.B.C.† with no. of whole asci in parentheses
	Cross 1 (67)	Cross 2 (50)	Cross 3 (55)	Cross 4, 5, 6, 7		
Sex to centromere	...	...	...	8.4	8.4	5.8 (1811)
al_2 to centromere	...	...	...	29.0	29.0	24.6 (191)
lys_1 to centromere	...	...	...	...	...	37.5 (64)
sex to al_2	35.4	32.0	30.9	37.4 "42.1"	33.5	30.4*
sex to lys_1	35.0	34.0	...	...	34.6	43.3*
al_2 to lys_1	4.4	18.0	...	...	9.8	12.9*
nd to centromere	...	...	...	14.6	14.6	...
nd to sex	24.6	26.0	14.6	23.0 "20.9"	22.0	...
nd to al_2	21.1	16.4	26.0	14.4 "34.2"	20.5	...

Cross 1: *nd* al_2 $+^{lys_1}$ *a*-F 3 × $+^{nd}$ $+^{al_2}$ lys_1 *A*-4545.

Cross 2: *nd* al_2 lys_1 *a*-64 × $+^{nd}$ $+^{al_2}$ $+^{lys_1}$ *A*-C8.

Cross 3: *nd* al_2 $+^{pe^m}$ $+^{fl}$ *A*-A 1 × $+^{nd}$ $+^{al_2}$ pe^m *fl a*-8743-21(13-7).

Cross 4: *nd* al_2 *a*-F 3 × $+^{nd}$ $+^{al_2}$ *A*-crassa 1 A.

Cross 5: *nd* al_2 lys_1 *a*-64 × $+^{nd}$ $+^{al_2}$ $+^{lys_1}$ *A*-C 8.

Cross 6: *nd* al_2 $+^{leu_1}$ *A*-A 1 × $+^{nd}$ $+^{al_2}$ leu_1 *a*-33757.

Cross 7: *nd* al_2 *A* (original) × $+^{nd}$ $+^{al_2}$ *a*-15300.

Numbers in parentheses indicate the number of single spores or asci isolated in each case.

Numbers in quotation marks stand for values estimated from recombination.

* Derived values.

† H.B.C. Houlaham, Beadle and Calhoun 1949.

An analysis of 24 whole asci and 172 randomly isolated spores gives the estimated map units between several genes shown in table 1. The gene is tentatively localized on the right arm of the mating type chromosome (linkage group I) about 15 map units from the centromere.

Dominance relationship

Heterocaryons involving *nd* and non-*nd* components were set up to determine dominance relationship (cf. BEADLF and COONRADT 1944; PONTECORVO 1946; BARRATT and GARNJOBST 1949). The results of such studies are summarized in table 2.

TABLE 2

Growth characteristics of heterocaryons on 40 cm growth tubes with minimal agar at 25° C.

Heterocaryon	Growth rate mm/hr	No. of tubes survived before stop	% of conidia *nd* : non-*nd* : heterocaryotic	Ratio of *nd*/non-*nd*
No. 1*	3.1–3.5	1.5	...	...
No. 2*	4.5	3	...	...
No. 3*	3.8–4.3	2.5	...	...
No. 4*	3.2	1.5	...	...
No. 5*	3.9–5.3	2.5	...	...
No. 6	2.6–3.2	8	32 : 42 : 26	0.8/1 (0.7/1–1/1)
No. 7**	3.7–4.2	0.5–1.5	...	...
No. 8†	3.6–4.5	19	49 : 27 : 24	1.8/1 (1.2/1–3.5/1)
No. 9	3.3–3.4	6	32 : 68 : 0.2	0.5/1 (0.4/1–0.8/1)
No. 10	3.4–4.0	6	24 : 30 : 46	0.8/1 (0.4/1–1.3/1)

No. 1: $+^{nd}\ +^{al_2}\ al_1\ +^{leu_1}\ +^{lys_1}\ +^{pan}$ *pab am A* with *nd* $al_2\ al_1\ leu_1\ +^{pab}\ +^{am}$ *A*–A 5.

No. 2: The same with *nd* $al_2\ +^{al_1}\ lys_1\ +^{pab}\ +^{am}$ *A*–C 7.

No. 3: The same with *nd* $+^{al_2}\ al_1$ *pan* $+^{pab}\ +^{am}$ *A*–10.

No. 4: $+^{nd}\ +^{al_2}\ +^{al_1}\ +^{leu_1}\ +^{lys_1}\ +^{pan}$ *am A* with *nd* $al_2\ +^{al_1}\ leu_1\ +^{am}$ *A*–5.

No. 5: The same with *nd* $+^{al_2}\ al_1$ *pan* $+^{am}$ *A*–10.

No. 6: The same with *nd* $al_2\ +^{al_1}\ lys_1\ +^{am}$ *A*–C 7.

No. 7: $+^{nd}\ al_2\ +^{al_1}\ lys_1\ +^{pan}$ *A*–D 3 with *nd* $+^{al_2}\ al_1\ +^{lys_1}$ *pan* A–10.

No. 8: *nd* $al_2\ +^{al_1}\ lys_1\ +^{pan}$ *A*–C 7 with $+^{nd}\ +^{al_2}\ al_1\ +^{lys_1}$ *pan A*–22.

No. 9: $+^{nd}\ +^{al_2}\ +^{lys_1}$ *pan* pe^m *fl A*–2 with *nd* $al_2\ lys_1\ +^{pan}\ pe^m\ +^{fl}$ *A*–13.

No. 10: $+^{nd}\ +^{al_2}\ al_1\ +^{lys_1}$ *leu*$_1$ $+^{pe^m}\ +^{fl}$ *A* with *nd* $al_2\ +^{al_1}\ lys_1\ +^{leu_1}\ pe^m$ *fl* *A*–13.

* There is probably selection for the *nd* nuclei, since the stopped frontiers appear mycelial in all cases.

** There is selection for the non-*nd* nuclei, since there is growth in heterocaryons made between the stopped frontiers and pantothenicless strains but not with lysineless strains.

† In one case a heterocaryon ceased to grow after thirteen transfers without significant change in conidia ratio.

As no *nd* strains and heterocaryons made of two *nd* strains survived more than three transfers in growth tubes at 25°C, those heterocaryons containing *nd* and non-*nd* components that survived six or more transfers with quite constant growth rates are very likely balanced heterocaryons which will survive indefinitely. The ratio of *nd* to non-*nd* homocaryotic conidia of such heterocaryons has been shown to vary from 0.4/1 to 3.5/1 in all cases. The tentative conclusion is therefore that one non-*nd* nucleus is somehow able to overcome the effect of 0.4 to 3.5 *nd* nuclei. Selection of either the *nd* or non-*nd* nuclear component in the heterocaryons was frequently observed. (See notes of table 2.)

Temperature responses

That the *nd* mutant is temperature sensitive is evident from the condition under which this mutant was picked up. The initial growth rate varies with temperature in the same manner as in the albino non-*nd* strain from which it was derived and in a way similar to the wild type *N. crassa* 1 A (RYAN, BEADLE and TATUM 1943). Besides growth rate the maximum amount of growth achievable is also a function of temperature (fig. 2, table 3). This is true not only of growth in tubes but also in liquid cultures. It is striking to see, however, that in liquid cultures the non-*nd* strain gives a set of growth curves similar to that of the *nd* strain (fig. 3). Nevertheless the limiting factors are different. After growth had stopped, bits of mycelium were transferred to new flasks. There was very little growth observed in the case of

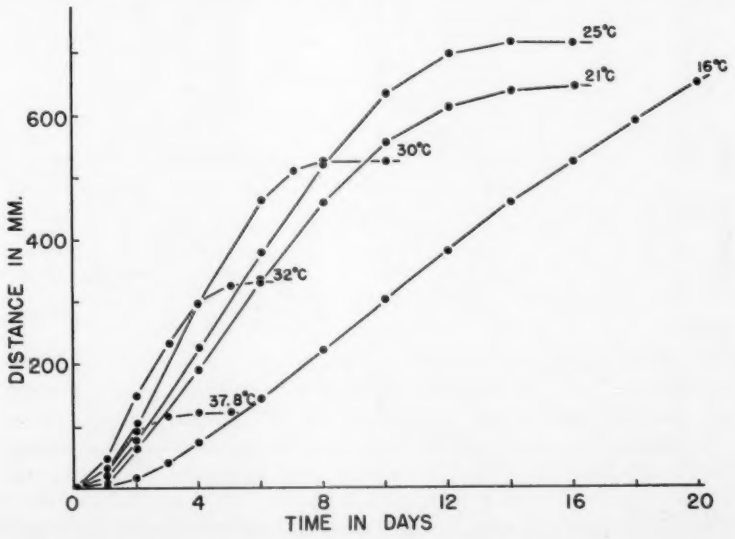


FIGURE 2.—Uninterrupted growth of *nd al*$_2$ *A*–A 1 (original single-spore culture) in growth tubes at different temperatures.

TABLE 3

Maximum distance reached by nd strains at different temperatures, the corresponding times and their reciprocals. D-Ca-pantothenate concentration 7.5 γ/15 ml for the pantothenicless strain.

Temperature	*nd al*$_1$ *pan A–10* (1st stock transfer)			*nd al*$_2$ *A–A 1* (1st stock transfer)		
	x (mm)	t (hr)	1/t	x (mm)	t (hr)	1/t
37.8°C	38	40	0.0250	46	49	0.0204
32°C	284	118	0.0084	198	121	0.0083
30°C	290	118	0.0084	262	247	0.0041
25°C	462	204	0.0049	434	356	0.0028
21°C	490	312	0.0032	390	344	0.0029
16°C	510	650	0.0015	410	667	0.0015

the *nd* strain, but full growth was observed in every case with the non-*nd* strain.

As t (table 3) is the time when the *nd* strain stops growing, 1/t is an arbitrary measurement of the rate of cessation of growth. When the logarithms of the value of 1/t (in percentage) which are taken from the smoothed curve of 1/t (in percentage) against temperature, are plotted against the re-

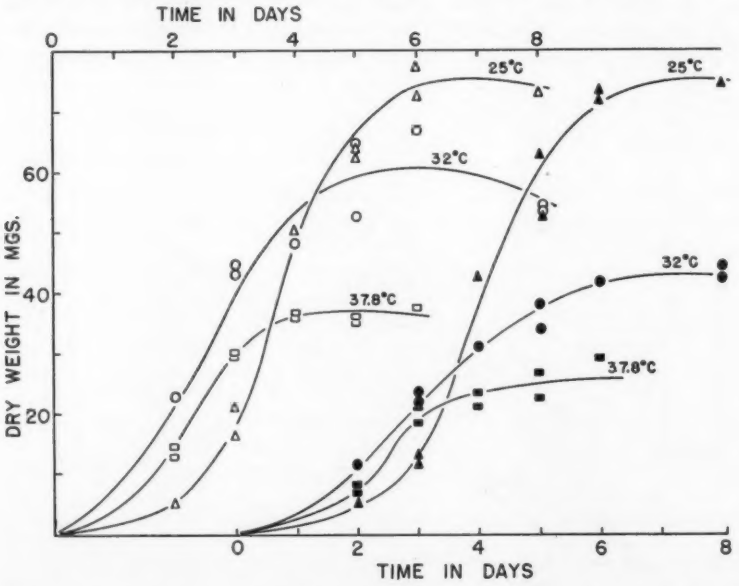


FIGURE 3.—Growth of *nd al*$_2$ *A*–A 1 (original single-spore culture) and $+^{nd}$ *al*$_2$ *A*–15300 in liquid cultures at different temperatures. Right set: *nd al*$_2$ *A*–A 1; left set: $+^{nd}$ *al*$_2$ *A*–15300.

ciprocal of absolute temperature (1/T) we have two straight lines intersecting at 33°C for the *nd al*$_1$ *pan* A–10 strain (fig. 4). The apparent energies of activation are 36,600 and 14,300 cal/mol for the upper and lower sections. A determination on another *nd* strain gives an intersection at 31°C and energies of activation of 38,300 and 16,000 cal/mol. The intersecting temperature seems to mean that below this temperature the cessation of growth is caused by the effect of the *nd* gene; while above this temperature cessation is primarily due to the effect of high temperature on Neurospora protoplasm.

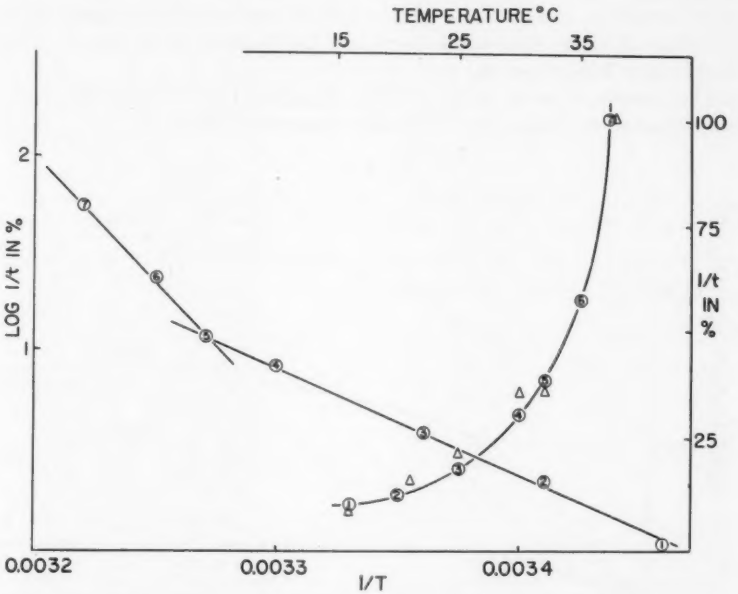


FIGURE 4.—Temperature characteristics of *nd al*$_1$ *pan A*–10. Right curve (upper right coordinates): 1/t in percentage against temperature °C; left curve (lower left coordinates): log 1/t in percentage, using values of 1/t in percentage taken from the right curve against 1/T. △ = experimental points; ○ = values taken from the right smoothed curve.

There are several reasons for this belief. Firstly, the optimum temperature for the rate of growth of the *nd* strain as well as the parental non-*nd* strain corresponds approximately to the intersecting temperature. Secondly, the cessation of growth of the *nd* strains at 32 and 37.8°C was usually temporarily reversed when they were transferred to 25°C. Thirdly, the growth of non-*nd* strains at 37.8°C did not usually occur at an uniform rate (fig. 1) and this was observed in some cases even at 32°C. Furthermore, the cessation of growth of the non-*nd* mold at higher temperature is reversed when the temperature is lowered to 25°C (RYAN *et al.* 1943).

The fact that the value of the apparent energy of activation of the lower section is of the order of ordinary enzymatic or biological reactions while that of the upper section is close to the value of heat inactivation of enzymes may suggest that the self-intoxicating process is not due to the destruction of certain enzymes during growth (SIZER 1943).

Growth rate and longevity

The growth rate of any Neurospora strain is certainly not a function of temperature alone. Different nutritional conditions affect the growth rate considerably. It would then be interesting to see what the relationship of growth rate to the maximal amount and the duration of growth is, if the temperature is kept constant.

The growth of an *nd* strain at 32°C in growth tubes with different concentrations of the salts of Fries medium is shown in figure 5.

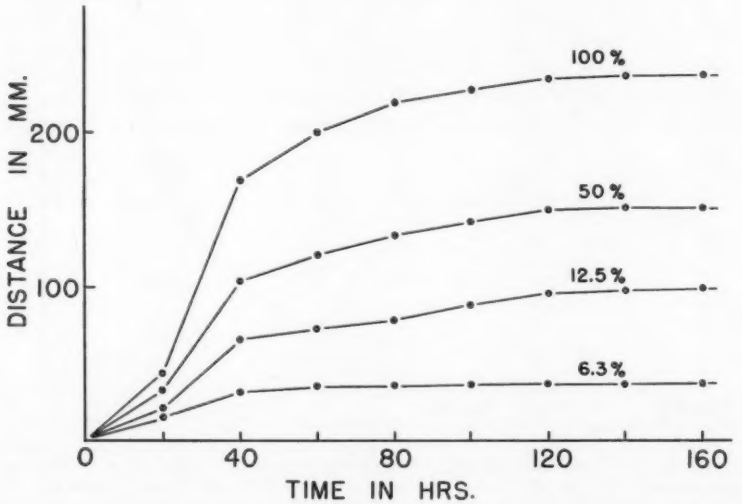


FIGURE 5.—Growth of *nd al₂ A*–A 1 (1st stock transfer) in tubes containing Fries agar of different salt concentrations at 32°C.

In connection with the test for the possible condition under which the *nd* strain might grow indefinitely, media of different composition and supplements were tried. This includes variation in condition of aeration, in salt concentrations, in biotin concentration, in sucrose concentration, in pH from 3.6 to 8.0, in the proportion of Na^+ and Ca^{++}, in media with yeast extract, casein hydrolysate, in Neurospora extract heated to boiling and with individual amino acids and water soluble vitamins placed in front of growing tips of old mycelium. Nothing was found to promote the growth of the *nd* strains considerably beyond that achieved under usual cultural conditions on minimal

medium. It was also observed that just as the results shown in figure 5, different nutritional conditions affect the initial growth rate and the amount of growth very much but less the duration of the growing period.

In passing it should be mentioned that *nd* extract had no injurious effect on the non-*nd* strain under the same experimental conditions.

Ageing under non-growing and other conditions

Experiments were performed to see if there is ageing in non-growing cultures. Transfers were made from the first, second, and the third stock transfers to four sets of 10×70 mm tubes labelled as the second, third and fourth stock transfers and let grow at 25°C for three days. A test in growth tubes was then made at 30°C, meanwhile these four sets were kept separately at 12, 25, 32, and 37.8°C for further tests, which were made at the tenth and the twentieth days after the first test. The results in table 4 show that ageing

TABLE 4

Ageing of nongrowing cultures of nd al$_2$ a-F 3 of different stock transfer (or age, kept at different temperatures as shown by the total amount of growth in mm reached at 30°C.

Stock transfer	Date when test made	Temperature under which stocks were kept			
		12°C	25°C	32°C	37.8°C
2nd stock transfer	1st day	257	265	301	304
	10th day	202	243	186	186
	20th day	211	264	225	8
3rd stock transfer	1st day	165	159	192	164
	10th day	147	154	142	110
	20th day	149	258	217	21
4th stock transfer	1st day	32	58	91	12
	10th day	24	25	59	20
	20th day	39	15	8	0

is going on in the non-growing cultures too. The process of ageing is however much slower compared with ageing during growth.

It has been mentioned that for some unknown reason the growth potential decreases tremendously under conditions of repeated subculturing. Table 5 illustrates the results of an experiment using growth tubes with side arms for taking samples along the tube. In the first column the figures stand for the distances from the inocula to the points where subcultures were taken. In the second column the distances reached by the subcultures in separate growth tubes are recorded. Sample A was taken at about 1 cm behind the growing frontier and sample B taken after the growth had stopped at the end of the tube which was 329 mm from the beginning. The values of the third column therefore represent the expected distance if the mold were not subcultured, and the final column the percentage of distance realized taking the values in the third column as one hundred percent.

TABLE 5

Effect of subculturing on the growth of nd al_2 A-A 1 1st stock transfer at 32°C.

Subculture taken from	Distance grown in mm after subculturing		Expected distance if not subcultured	Percentage realized	
	Sample A*	Sample B*		Sample A	Sample B
48 mm	120	61	329 − 48 = 281	43	22
96 mm	62	35	329 − 96 = 233	27	15
150 mm	24	20	329 − 150 = 179	13	11
199 mm	19	0	329 − 199 = 130	15	0
252 mm	3	0	329 − 252 = 77	4	0

*See text.

Rejuvenation through heterocaryosis

It has been shown that balanced heterocaryons can be established in certain cases. It is therefore suggested that if the ageing process were due to the accumulation of some toxic substances, or the exhaustion of some essential substances, one would naturally expect that the *nd* strain might be rejuvenated if pure strains were isolated from those balanced heterocaryons which show no cessation of growth. For this purpose heterocaryons between microconidiating cultures *nd* al_2 lys_1 $+^{pan}$ pe^m *fl* A–13 and $+^{nd}$ $+^{al2}$ $+^{lys1}$ *pan* pe^m *fl* A–2 (No. 9, table 2) were used. Experiments confirmed the findings of BARRATT and GARNJOBST (1949), that there is at most 0.5 percent of heterocaryotic microconidia which give rise to colonies on minimal agar plates. Conidia were plated on agar with 0.05 percent sorbose and 0.01 percent sucrose supplemented with an optimal concentration of DL-lysine (0.5 mg/15 ml) or (+)-Ca-pantothenate (7.5γ/15 ml) for the two types of homocaryotic colonies. Individual colonies were transferred directly to growth tubes supplemented with optimal lysine. Conidia from pure cultures of the non-*nd* strain and the first stock transfer of the *nd* strain were also plated and transferred parallel to the experimentals. Conidia are so few and may be so inviable in the third stock transfer of the *nd* strain (practically none are present in the fifth stock transfer) that corresponding controls were taken directly from the pure cultures instead of from single conidial colonies. Ten colonies were tested in each case. The results are shown in table 6.

The results are striking enough to show that no matter how "old" the *nd* strain is, it will rejuvenate through heterocaryon formation to about the same extent. All *nd* isolates from heterocaryons exceed the corresponding pure cultures in the amount of growth and approach or equal that reached by the original culture, which under the same conditions gave 552 mm of growth in two determinations. The corresponding non-*nd* (and *pan*) isolates were tested in growth tubes supplemented with optimal Ca-pantothenate. The means of the growth rates at 30°C are 4.1 ± 0.4, 4.1 ± 0.3 and 4.0 ± 0.3 mm/hr for non-*nd* isolates from heterocaryons made between the first and the fifth stock transfer and a non-*nd* culture, and from the non-*nd* culture

TABLE 6

Rejuvenation through heterocaryosis. Total amount of growth in mm reached by nd isolates from balanced heterocaryons made between different stock transfers of nd al_2 lys_1 $+^{pan}$ pe^m fl A-13 and $+^{nd}$ $+^{al_2}$ $+^{lys_1}$ pan pe^m fl A-2 and corresponding colonies or inocula from pure cultures of the nd component.

nd (and lys_1) colonies or inocular from	1st stock transfer		3rd stock transfer		5th stock transfer	
	Colonies from hetero-caryon	Colonies from pure culture	Colonies from hetero-caryon	Inocula from pure culture	Colonies from hetero-caryon	Inocula from pure culture
Approximate total amount of growth in mm at 32°C	380	305	440	250	605	0
	455	325	605	260	670	0
	390	350	375	250	600	...
	410	325	410	250	495	...
	370	140	620	...	510	...
	305	580	500	...	685	...
	605	535	615	...	470	...
	630	0	665	...	255	...
	605	55	300	...	600	...
	0	324	0	...	400	...
Mean (disregarding zero readings)	461	380	505	252	529	0

itself. It is, however, a peculiarity of this non-*nd* culture that even at temperatures as low as 30°C the growth rate is as a rule not constant.

Rejuvenation through crossing

Similar experiments were done using the sexual process instead of asexual heterocaryon formation. Crosses were made between the different stock transfers (or ages) of the same strain *nd* al_2 *a*–F 3, and a single non-*nd* strain, *N. crassa* 1 A. Ascospores were isolated, activated and tested in growth tubes. The results were analogous to those in the experiments with heterocaryons, *i.e.*, all of the *nd* segregants were rejuvenated to the same extent no matter what growth phase or which stock transfer was used in the cross.

From the experiments on rejuvenation through heterocaryosis and through crossing it seems reasonable to suspect that there might be a difference between reciprocal crosses. Crosses were therefore made using one or the other strain as the protoperithecial parent or the conidial parent. Spores were isolated and tested and all the *nd* segregants were found to be rejuvenated to the same extent. The negative result may be due to the fact that the difference in the amount of cytoplasm contributed by the conidium and by the trichogyne is not large enough to be demonstrated by reciprocal crossing.

It was thought that if the cytoplasm of the non-*nd* parent that had been used in these crosses was responsible for the rejuvenation, a cross of two aged *nd* strains would not be expected to yield rejuvenated spores. Repeated trials to cross *nd* to *nd*, old and young, using Westergaard's agar, plain or

supplemented with yeast extract and casein hydrolysate, corn meal agar, normal and very low concentration of sucrose (0.01 percent) and at different temperatures, always led to failure. A cross was also made between *nd* al_2 a–49 and a heterocaryon consisting of the amycelial strain $+^{nd}$ $+^{al2}$ $+^{lys1}$ *am* A–422–6 and *nd* al_2 lys_1 $+^{am}$ A–C 7. Five asci were isolated which gave five to eight germinated spores per ascus upon activation. All of them proved to be *nd* and grew as long as the parental cultures. Crosses with heterocaryons were therefore successful and *nd* nuclei of different mating types were shown to be able to undergo syngamy and meiosis.

DISCUSSION

A discussion of the nature of the *nd* mutant is necessarily based on indirect evidence as described above.

Evidence such as rejuvenation through heterocaryosis and crossing between two *nd* strains if one of them is in the heterocaryotic condition leads one to the belief that ageing is going on in the cytoplasm.

A number of hypotheses may be proposed to explain this ageing process and rejuvenation through heterocaryosis as well as crossing. Something injurious might be accumulated during growth, in this case rejuvenation would mean the dilution or destruction of the toxic substance. Something essential might be exhausted during growth, in this case rejuvenation would mean the introduction of this substance into the *nd* ascospore or conidium.

In liquid medium we get about 80 mg of mycelium in each culture inoculated from the original single-spore culture. A calculation of the total amount of mycelium obtainable from such a single-spore culture and the amount of substance that might be carried along in an ascospore or conidium suggests that the later hypothesis is less likely. It requires the substance to be at least a hundred times as active as biotin to support that amount of growth (MELIN and NORKRANS 1948).

The hypothesis of a defective enzyme is also less acceptable; if so it seems that the mutant would grow at a lower but uniform rate instead of high initial but ever-decreasing rate.

The hypothesis of the accumulation of a self-intoxicating substance is more likely though there is no direct experimental support. Besides rejuvenation it explains the temperature response by assuming that temperature affects the rate of synthesis of the *nd* substance more than the rest of the anabolic activities, consequently the higher the temperature the smaller the amount of growth.

There still remain some unexplained facts. It seems that different nutritional conditions affect reactions determining the rate of growth but leaving the synthesis of the *nd* substance relatively unaffected so that we have a picture like figure 5 when the *nd* strain is subject to different nutritional conditions. The whole story is of course complicated by the very complex mode of growth (RYAN *et al.* 1943) by which any substance may accumulate in the growing frontier of the mycelium. Facts like the tremendous decrease in

growth potential after subculturing are entirely unexplained. The conclusion of an accumulation of self-intoxicating substance is therefore only tentative until the substance is isolated or at least its action demonstrated in vitro.

SUMMARY

A mutant strain, called natural death or *nd*, was induced in *Neurospora crassa* by ultraviolet radiation and isolated by means of the sorbose plating technique. It is characterized by its ever-decreasing growth potential under all nutritional conditions and the irreversible cessation of growth either on agar surface or in liquid culture. The responsible gene has been localized on the right arm of the mating type chromosome about 15 map units from the centromere. The *nd* gene is recessive to its wild allele, because balanced heterocaryons that seem to grow indefinitely are formed with non-*nd* strains and the ratio of *nd* to non-*nd* conidia is not far from unity.

The mutant is temperature-sensitive in the sense that the higher the temperature, the higher the growth rate and lower the amount of growth. However, if the temperature is kept constant and the growth rate altered by changing the nutritional condition, then the higher the growth rate, the higher the amount of growth. Ageing not only goes on during growth but also under non-growing conditions.

The aged strain can be rejuvenated through heterocaryosis with non-*nd* strains, through crossing to non-*nd* strains, and through crossing to another *nd* strain which is in a heterocaryotic condition. In each case the degree of rejuvenation is independent of the age of the *nd* strain used. Direct crossing of one *nd* strain to another has not yet been possible.

A hypothesis of cytoplasmic ageing, assuming the accumulation of a self-intoxicating substance in the growing frontier is proposed.

ACKNOWLEDGMENT

The author wishes to thank PROFESSORS F. J. RYAN and K. C. ATWOOD for their suggestions and helpful criticism during the course of the investigation and in the preparation of the manuscript. The author is indebted to DR. K. C. ATWOOD and DR. R. W. BARRATT for their amycelial and microconidiating cultures and to DRS. S. EMERSON and N. H. HOROWITZ for helpful criticism during the preparation of this manuscript.

LITERATURE CITED

BARRATT, R. W., and L. GARNJOBST, 1949 Genetics of a colonial microconidiating mutant strain of *Neurospora crassa*. Genetics **34**: 351–369.

BEADLE, G. W., and V. L. COONRADT, 1944 Heterocaryosis in *Neurospora crassa*. Genetics **29**: 291–308.

BONNER, D., 1946 Biochemical mutations in Neurospora. Cold Spring Harbor Symp. Quant. Biol. **11**: 14–24.

HOROWITZ, N. H., 1948 The one gene one enzyme hypothesis. Genetics **33**: 612–613.

1950 Biochemical genetics of Neurospora. Advances in Genetics **3**: 33–71.

HOULAHAN, M. B., G. W. BEADLE and H. C. CALHOUN, 1949 Linkage studies with biochemical mutants of *Neurospora crassa*. Genetics **34**: 493–507.

MELIN, E., and B. NORKRANS, 1949 Determination of biotin in beet molasses with *Neurospora crassa* Shear and Dodge as a testing organism. Plant and Soil **1**: 2–10.

PONTECORVO, G., 1946 Genetic systems based on heterocaryosis. Cold Spring Harbor Symp. Quant. Biol. **11**: 193–201.

RYAN, F. J., G. W. BEADLE and E. L. TATUM, 1943 The tube method of measuring the growth rate of Neurospora. Amer. J. Bot. **30**: 784–799.

SIZER, J. W., 1943 Effect of temperature on enzyme kinetics. Adv. in Enzymol. **3**: 35–62.

TATUM, E. L., R. W. BARRATT and V. M. CUTTER, JR., 1949 Substances inducing a colonial paramorphic effect in Neurospora and Syncephalastrum. Amer. J. Bot. **35**: 803.

INFORMATION FOR CONTRIBUTORS

Contributors to GENETICS may be in the field of genetics proper, or in any scientific field, if of such a character as to be of primary genetic interest.

The length of manuscripts will be limited to twenty-five printed pages (about twelve thousand words) except by special vote of the Editorial Board. In case unusually extensive tabular matter cannot be printed, it will be kept on file on request, provided two copies are furnished by the author.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to cover the cost of reproducing colored and halftone plates and of printing expensive tables.

Authors should prepare their manuscripts so as to conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a summary. In case of "Acknowledgments," these should follow the "Summary." Footnotes should be avoided in the text whenever possible and their content be inserted, in parentheses, after the appropriate sentence. Footnotes to tables should be marked with an asterisk, dagger, or other symbol so as not to be confused with the figures in tables. Legends for figures and plates should be type-written separately from the illustrations. In addition to the original illustrations photographic copies should accompany the manuscript. Tables also should be type-written separately. The metric system should be used whenever possible.

Galley proofs and page proofs will be sent to authors, except in case of authors resident overseas who, in general, will receive galley proofs only. Authors should leave forwarding directions whenever they are to be away from the address sent with the manuscript.

GENETICS furnishes 50 reprints, without covers, free. Covers and additional reprints may be secured at cost.

Manuscripts and editorial correspondence should be addressed to the Editor of GENETICS, Department of Zoology, University of California, Berkeley 4, Calif.

GENETICS Vol. 36 No. 2 MARCH, 1951

CONTENTS OF PRECEDING ISSUES

JULY, 1950

CONGER, ALAN D., and NORMAN H. GILES, JR., The cytogenetic effect of slow neutrons.
BRIEGER, F. G., The genetic basis of heterosis in maize.
BURNHAM, C. R., Chromosome segregation in translocations involving chromosome 6 in maize.
GERSTEL, D. U., Self-incompatibility studies in Guayule II. Inheritance.

SEPTEMBER, 1950

JONES, DONALD F., The interrelation of plasmagenes and chromogenes in pollen production in maize.
ROGERS, JOHN S., The inheritance of photoperiodic response and tillering in maize-teosinte hybrids.
ROGERS, JOHN S., The inheritance of inflorescence characters in maize-teosinte hybrids.
HANSON, W. D., and H. H. KRAMER, The determination of linkage intensities from F_2 and F_3 genetic data involving chromosomal interchanges in barley.
HUGHES, MORRIS B., and ERNEST B. BABCOCK, Self-incompatibility in *Crepis foetida* (L.) subsp. *rhoeadifolia* (Bieb.) Schinz et Keller.
KOMAI, TAKU, MITSUSHIGE CHINO and YASUSI HOSINO, Contributions to the evolutionary genetics of the lady-beetle, Harmonia. I. Geographic and temporal variations in the relative frequencies of the elytral pattern types and in the frequency of elytral ridge.

NOVEMBER, 1950

NEWCOMBE, H. B., and M. H. NYHOLM, The inheritance of streptomycin resistance and dependence in crosses of *Escherichia coli*.
CROW, JAMES F., and WILLIAM C. ROBERTS, Inbreeding and homozygosis in bees.
FULLER, JOHN L., CLARICE EASLER and MARY E. SMITH, Inheritance of audiogenic seizure susceptibility in the mouse.
BRILES, W. E., W. H. MCGIBBON and M. R. IRWIN, On multiple alleles affecting cellular antigens in the chicken.
Abstracts of papers presented at the 1950 Meetings of the Genetics Society of America.
INDEX TO VOLUME 35.

JANUARY, 1951

DAVIS, BRADLEY M., Eduard Strasburger.
DUNN, L. C., and S. GLUECKSOHN-WAELSCH, On the origin and genetic behavior of a new mutation (t^3) at a mutable locus in the mouse.
PAVAN, C., A. R. CORDEIRO, N. DOBZHANSKY, TH. DOBZHANSKY, C. MALOGOLOWKIN, B. SPASSKY and M. WEDEL, Concealed genic variability in Brazilian populations of *Drosophila willistoni*.
WHITE, M. J. D., A cytological survey of wild populations of *Trimerotropis* and *Circotettix* (Orthoptera, Acrididae). II. Racial differentiation in *T. sparsa*.
RUTMAN, ROBERT J., The inheritance of rates of methionine incorporation by rats and the relations to growth.
GARDNER, ELDON J., and GERALD H. STOTT, Genes producing a maternal effect and modifiers of tumorous head in "wild" and tumor bearing stocks of *Drosophila melanogaster*.
NEEL, JAMES V., and MARY M. HANIG, The inheritance and frequency of the MNS factors in the American Negro.
BELLAMY, ALBERT W., and MARION L. QUEAL, Heterosomal inheritance and sex determination in *Platypoecilus maculatus*.

SINGLE NUMBERS $1.50 ANNUAL SUBSCRIPTION $8.00
obtainable from
BROOKLYN BOTANIC GARDEN, BROOKLYN 25, NEW YORK